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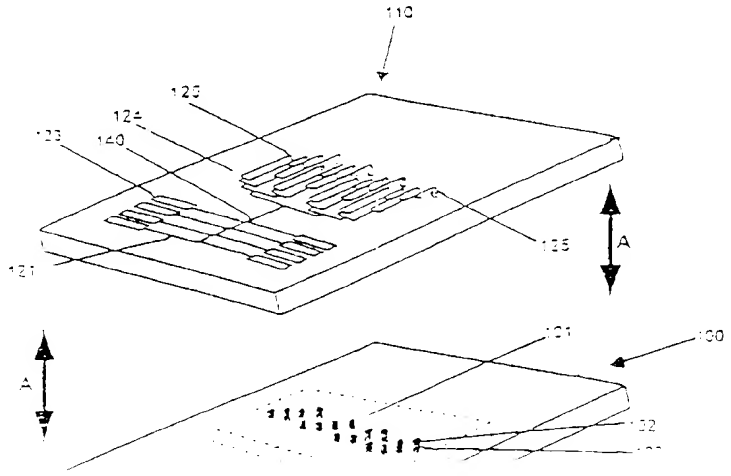
(54) Title: MICROFLUIDIC DEVICES AND METHODS



(57) Abstract: Embodiments of the invention are directed to microfluidic devices. In one embodiment, a microanalysis [illegible]

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MICROFLUIDIC DEVICES AND METHODS

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional patent application nos. 60/184,381 filed February 23, 2000 and 60/225,999 filed August 16, 2000. This application is also being filed on the same day as U.S. non-provisional application no. _____ entitled "Chips With Elevated Sample Surfaces" by Pierre F. Indermuhle et al. (Attorney Docket No. 020144-000810). All of the above provisional and non-provisional patent applications are herein incorporated by reference in their entirety for all purposes and are all assigned to the same assignee as the present application.

BACKGROUND OF THE INVENTION

Conventional methods for performing high throughput mass spectrometric (MS) protein identification employ either 2D-PAGE technology or various modes of multidimensional chromatography. 2D-PAGE is commonly used in proteomics (i.e., the study of proteins). In a typical 2D-PAGE process, 3000 to 5000 different proteins can been separated. After separating, spots of the separated proteins can be cut out and analyzed using mass spectrometry.

Conventional 2D-PAGE technology, however, has a number of drawbacks. Drawbacks include low sensitivity (e.g., 1 ng protein detection limit with silver staining), the limited range of proteins that can be analyzed, (membrane proteins, high molecular, low molecular proteins are underrepresented), and low sample throughput. The low sample throughput is due to the labor and time intensiveness of this method. For example, 2D-PAGE systems allow for the processing of only 10 gels in two days per system. In order to take advantage of MS, additional equipment (e.g., robotic gel spot cutters and digest workstations) is required. Besides being complex and costly

chromatography techniques may offer faster analysis cycles. However these methods have limitations including low detection limits and the limited scope of proteins that may

be analyzed (e.g., due to solution condition limitations imposed by the selected chromatographic method).

Embodiments of the invention address these and other problems.

SUMMARY OF INVENTION

One embodiment of the invention is directed to a microanalysis chip comprising a body defining at least one transfer-separation channel including a channel bottom having a bottom opening, the transfer-separation channel terminating in a discharge aperture.

Another embodiment of the invention is directed to a method for chemically affecting a sample, the method comprising: providing a microanalysis chip including a body having a transfer-separation channel with a channel bottom having a bottom opening; inserting a pillar into the bottom opening such that a sample supported by the pillar communicates with the transfer-separation channel; and passing a reagent fluid into the transfer-separation channel in order for the reagent fluid to come in contact with the sample to chemically affect the sample.

Another embodiment of the invention is directed to a dispenser assembly comprising: a dispenser chip including a dispenser body including a vertical channel; and a sample chip having a base and a sample structure, the sample structure comprising a pillar and a sample surface, wherein the vertical channel of the dispenser chip is cooperatively structured to receive the pillar.

Another embodiment of the invention is directed to a microfluidic chip comprising: a body having a bottom surface; a plurality of discharge apertures; and a plurality of transfer-separation channels in the body, each transfer-separation channel defined by a channel bottom with a bottom opening, and having a portion upstream of the bottom opening and a portion downstream of bottom opening, and wherein each transfer-separation channel terminates at one of the discharge apertures.

Another embodiment of the invention is directed to a microfluidic

[illegible] defined by a channel bottom with a bottom opening, and having a portion upstream of the bottom opening and a portion downstream of bottom opening, and wherein [illegible]

discharge apertures; and a sample chip comprising a base including a non-sample surface and a plurality of sample structures having a plurality of sample surfaces.

Another embodiment of the invention is directed to a method of processing an analyte, the method comprising: processing an analyte on a sample surface on an sample chip; transferring the processed analyte through a transfer-separation downstream of the sample surface, wherein the transfer-separation channel is in a microfluidic chip above the sample chip; and analyzing the processed analyte downstream of the sample surface.

Another embodiment of the invention is directed to a microfluidic chip comprising: a body having a bottom surface; and a plurality of vertical channels in the body, wherein each opening is cooperatively structured to receive a pillar of a sample chip.

Another embodiment of the invention is directed to a method of processing analytes, the method comprising: inserting a plurality of sample surfaces into a plurality of vertical channels in a dispenser chip, wherein the plurality of sample surfaces are on pillars of a sample chip; depositing a plurality of liquid samples on the sample surfaces while the sample surfaces are in the vertical fluid channels; binding analytes from the plurality of liquid samples to the sample surfaces; withdrawing the sample surfaces from the vertical fluid channels; inserting the plurality of sample surfaces into a plurality of openings in a microanalysis chip so that the plurality of sample surfaces are in communication with a plurality of transfer-separation channels in the microanalysis chip; and processing the analytes using reagents flowing through the transfer-separation channels while the analytes are bound to the sample surfaces.

Another embodiment of the invention is directed to an analysis system comprising: an analysis assembly comprising (i) a microanalysis chip comprising a body comprising at least one transfer-separation channel defined by a channel bottom having a bottom opening, the transfer-separation channel terminating in a discharge aperture, and (ii) a sample chip having a plurality of sample surfaces; and an analysis device adapted to receive an analyte from the discharge aperture

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view of a dispenser chip spaced from a chip holder engaging a sample chip.

FIGS. 2(a)-2(b) show cross-sections of chips including pillars.

FIGS. 3 and 4 show cross-sectional views of pillars with affinity structures.

FIG. 5 shows a perspective view of an array of pillars.

FIGS. 6(a) to 6(b) show cross-sections of pillars.

FIGS. 6(c) to 6(h) show perspective views of different types of pillars that may be on a base of a chip.

FIGS. 6(i) to 6(l) show cross-sections of pillars.

FIG. 7 shows a perspective view of a dispenser.

FIG. 8 shows a perspective view of a chip embodiment.

FIG. 9 shows a perspective view of an assembly embodiment.

FIGS. 10-12 shows cross-sectional views of assembly embodiments.

FIG. 13 is a close-up view of a liquid sample on a sample surface of a pillar.

FIG. 14 shows a cross-sectional view of an assembly embodiment.

FIGS. 15 to 16 show cross-sectional views of assembly embodiments.

FIGS. 17(a) to 17(d) show cross-sectional views of an assembly embodiment including a chip with a pillar having a concave side surface.

FIG. 18 is a perspective view of a microanalysis chip spaced from a sample chip engaged to a chip holder.

FIG. 19 is a top view of a microanalysis chip.

FIG. 20 is a top view of a microanalysis chip with horizontal fluid channels being shown by dotted lines.

FIG. 21 is a perspective view of a portion of a microanalysis chip with reservoirs being shown by dotted lines.

FIG. 23 is a perspective view of a portion of an analysis assembly with a chromatography/retention zone.

FIG. 24 is a perspective view of a portion of an analysis assembly with a seal.

FIG. 25 is a side cross-sectional view of an analysis assembly with a seal.

FIG. 26 is a perspective view of a portion of an analysis assembly with a lid.

FIGS. 27 and 28 are top views of analytical assemblies with a lid.

FIGS. 29 to 34 show cross-sectional views of the discharge apertures of various microanalysis chip embodiments.

FIG. 35 shows a schematic drawing of a system embodiment of the invention.

DETAILED DESCRIPTION

Embodiments of the invention find utility in the post-genome era of functional genomics or proteomics to decipher the complex interplay among various biological molecules (e.g., genes, gene products, metabolites, proteins) in biological systems. They utilize array type devices for analyte isolation along with microfluidic devices that may have micropurification capability. The strengths of array-type devices and the strengths of microfluidic type devices may be combined to perform multiple processes in parallel and in an integrated manner.

In commercial settings, embodiments of the invention may be used in the pharmaceutical industry for, e.g., proteomic studies. Using embodiments of the invention, potential drug candidates can be discovered or can be verified as being therapeutic. Embodiments of the invention may also be used as diagnostic tools in a clinical setting for staging or disease progression. They may find use in environmental analyses to track and identify contaminants. In academic research environments, embodiments of the invention may be employed in basic biology or medical research. Embodiments of the invention can be used to characterize and quantify analytes ranging from small organic molecules (e.g., [illegible] metabolites, particles [illegible]

Embodiments of the [illegible] include microfluidic assemblies. Typical microfluidic assemblies may include microfluidic devices that are used in conjunction [illegible]

microanalysis chips. In a typical embodiment, a dispenser chip and a microanalysis chip may be sequentially interfaced with a sample chip comprising sample structures in an array. Each sample structure may include a pillar and a sample surface on the pillar. Openings at the bottoms of the dispenser and the microanalysis chip can be structured to receive the pillars of the sample chip.

In some embodiments, the dispenser chip may deposit fluids on the sample surfaces of a sample chip. The deposited fluids may process analytes (or analyte derivatives) on the sample surfaces. The microanalysis chip may be also used to process analytes (or analyte derivatives) on the sample surfaces of a sample chip. In addition, the microanalysis chip can prepare analytes for subsequent analysis, and/or transfer to an analysis device. Typically, the prepared analytes may be transferred downstream of the sample surfaces through transfer-separation channels in the microanalysis chip. The prepared analytes may then be discharged from the transfer-separation channels to an analysis device where a desired analysis can take place.

For purposes of illustration, some of the specifically described embodiments below refer to using a dispenser chip or a microanalysis chip to process analytes such as proteins on the sample surfaces of a sample chip. However, it is understood that analyte derivatives may also be processed on the sample surfaces. Analyte derivatives include previously processed analytes. Specific examples of analyte derivatives may include subunits or subunit mixtures of analytes that have been previously cleaved, or analytes that have been derivatized with another substance. For example, an analyte may be a protein while an analyte derivative may be a derivatized protein or a mixture of protein subunits.

The assemblies and chips according to embodiments of the invention can be used in a mass spectrometric analysis to identify and characterize analytes such as proteins. For example, a dispenser chip may be used to deposit liquids containing proteins on an array of sample surfaces of a sample chip. The deposited liquids may comprise complex liquid samples such as cell lysate and bodily fluids. Proteins in the liquid samples bind to the array [illegible]

so that they are suitable for an analysis such as mass spectrometry. For example, the dispenser chip and/or an analysis chip may be interfaced with the sample chip containing [illegible]

such as liquid reagents on the sample surfaces. The processing fluids can be used to process the proteins bound to the sample surfaces of the sample chip. Exemplary processing fluids include reagents that can cleave, unfold, or derivatize analytes. Reagents may also include fluids that can separate an immobilized analyte or an analyte derivative from a sample surface. After separation, the analyte or the analyte derivative may be transferred downstream of the sample surface.

Capturing analytes from liquids on a capture array of sample surfaces to isolate the analytes and process them on the sample surfaces provides advantages over conventional analysis methods. For example, such advantages include the isolation of proteins under native conditions, faster analysis times, and the selective enrichment of low abundance proteins.

When the proteins are on the sample surfaces, they may be processed in any suitable manner. For example, the processing fluids dispensed by the dispenser chip or the microanalysis chip may be used to help react, purify, concentrate, and/or separate proteins or protein derivatives so that they are suitable for a mass spectrometry analysis. For instance, a reagent can be delivered to a sample surface to cleave a protein to form a peptide mixture. Solutions (e.g., aqueous, organic, acidic) may be delivered to the sample surface and added to the peptide mixture so that the resulting product can be analyzed by a mass spectrometer.

After processing, an analysis assembly comprising the microanalysis chip and the sample chip may be transported to a mass spectrometer using a translation stage system (e.g., an x-y-z axis positioning system). The processed proteins can be transported in transfer-separation channels in the microanalysis chip to discharge apertures in the microanalysis chip. The processed proteins may then be discharged from the microanalysis chip along with any carrier fluids. After discharging, the processed proteins may be received by a sampling orifice of a mass spectrometer. Once received, the mass spectrometer can create appropriate mass spectra for the received protein mixture to characterize or quantify the received mixture.

Any number of mass spectrometric or spectroscopic techniques may be

mass spectrometry (TSP MS), or matrix assisted laser desorption ionization mass spectrometry (MALDIMS). For mass analysis, any type of analyzer may used.

Exemplary analyzers may include quadrupole, time-of-flight, ion trap, and Fourier transform ion cyclotron resonance analyzers.

The use of the assemblies with mass spectrometric detectors provides high sample throughput, reduced cost per analysis, reduced reagent usage, minimized contamination, and reduced sample losses. The reduction in sample losses results in an increase of the overall sensitivity of the system. Both qualitative and quantitative data may be gathered on particular molecules from various samples (e.g., cell lysate, body fluids, etc.) using embodiments of the invention. The data may be compared in differential type experiments to identify and/or characterize changes of specific molecules as a function of state (e.g., normal vs. disease samples).

I. Dispensing assemblies

The dispensing assemblies according to embodiments of the invention may include a sample chip that has sample surfaces and a dispenser that dispenses liquids on the sample surfaces. To reduce the potential for cross-contamination between adjacent sample surfaces, the sample surfaces may be elevated with respect to a non-sample surface of the sample chip. Typically, the sample surfaces are on pillars that raise the sample surfaces above the non-sample surface.

In some embodiments, a plurality of liquids may be supplied to the fluid channels in a dispenser. The liquids supplied to the different fluid channels may be the same or different and may contain the same or different components. For example, each of the liquids in respective fluid channels may include different reagents. The dispenser may dispense the liquids on the sample surfaces of a sample chip in parallel. Discrete deposits of liquid samples may be left on the sample surfaces. The liquid samples may contain analytes that are to be bound to the sample surface. Alternatively or additionally, the liquid samples may comprise reagents that are used to process analytes at the sample surfaces.

The liquid samples

antigens, proteins, lipids, oligonucleotides, oligosaccharides, steroids, cholesterols, etc. Other analytes may include cell organelles such as golgi, and chemical compounds such

for example, combinatorial chemistry techniques. Reagents may include substances that can react with other components on the sample surfaces. More specifically, suitable reagents may include biological or chemical entities that can process components at the sample surfaces. For instance, a reagent may be an enzyme or other substance that can cleave unfold, cleave, or derivatize the proteins at the sample surface. Suitable liquid media in the liquid samples include solutions such as buffers (e.g., acidic, neutral, basic), water, organic solvents, etc.

The liquids from which the liquid samples are obtained may be man-made or naturally occurring. For example, the liquids may be derived from, or be, biological fluids such as blood, urine, plasma, cerespinal fluid, tears, saliva, biopsy samples, etc.

FIG. 1 shows a dispensing assembly including a dispenser chip **110** and a chip assembly **100** comprising a sample chip **105** and a chip holder **106**. The dispensing assembly **110** and the chip assembly **100** may move in the direction of the arrows **A** to engage each other. The dispenser chip **110** may have a structure similar to that of a microanalysis chip. However, dispenser chips typically do not have discharge apertures downstream of the sample surfaces of the sample chips.

The chip holder **106** has a depression that receives the sample chip **105**. The sample chip **105** may have sample structures on a base **105a**. Each sample structure may comprise a pillar **103** and a sample surface **102**. When the sample chip **105** is in the depression, the top surface of the base **105a** of the sample chip **105** can be substantially coplanar with the top surface of the chip holder **106**. A bar code **116** or other identifier may be present on the chip holder **106**.

The dispenser chip **110** includes a plurality of vertical fluid channels **125** that receive the pillars **101** of a sample chip **105**. In some instances, the vertical fluid channels **125** may be referred to as "wells". In the dispenser chip **110**, the vertical fluid channels **125** are in communication with a distribution network **124**. The distribution network **124**, in turn, communicates with a main delivery channel **140**. A plurality of delivery channels **121** couples reservoirs **123** to the main delivery channel **140**. The reservoirs **123** may contain liquids such as reagents and analyte containing fluids.

For example, in some embodiments, the delivery channels **121** may communicate directly with the reagent distribution network **124** without using a main delivery channel **140**.

The fluid channels in the dispenser chip and in the microanalysis chip (described in further detail below) may have any suitable characteristics. In some embodiments, a fluid channel may be defined by three sides. For example, a fluid channel may be defined by two side surfaces and a bottom surface. These surfaces in combination can form an open fluid channel. A fluid channel may also be defined by four sides. For example, a flat plate may be over an open fluid channel to form a closed fluid channel. The fluid channels may have a circular or polygonal (e.g., rectangular) cross-sectional geometry. Regardless of the particular cross-sectional geometry of the fluid channels, the fluid channels may be closed or open to any desired degree as long as fluids are allowed to pass through the fluid channels. In addition, the dimensions of the fluid channels may vary. For example, a cross-sectional dimension of a fluid channel in the dispenser may be between about 0.1 to 500 microns (e.g., about 0.1 to 100 microns). In other embodiments, it may be from about 1.0 to about 500 microns (e.g., about 1 to about 100 microns).

Illustratively, a sample chip **105** having sample structures containing pillars **101** and sample surfaces **103** is interfaced with a dispenser chip **110**. The sample chip **105** may be brought in contact with the dispenser chip **110** and the pillars **101** of the sample chip **105** are inserted into the vertical fluid channels **125** of the dispenser chip **110**. Liquids in the reservoirs **123** flow through the delivery channels **121**, through the common delivery channel **140**, through the distribution network **124**, and to the desired vertical fluid channel **125**. The liquids pass from the vertical fluid channels **125** to the sample surfaces **103** of the pillars **101**.

The liquids that contact the sample surfaces **103** may contain analytes that are immobilized on the sample surfaces **103**. As explained in detail below, the sample surfaces **103** may be formed by affinity structures that selectively bind particular analytes and thus isolate them from other components in the liquids. Alternatively or additionally, the liquids that contact the sample surfaces **103** may be reagents that may be delivered to the sample surfaces **103** to unfold, derivatize, or cleave the analytes or analyte derivatives (e.g., analytes previously cleaved analyte subunits) that are on the sample surfaces 103

[illegible] liquids, but all of the subsequent [illegible] are performed on an intact analyte. For example, reagents may be deposited [illegible] sample surfaces **103** to prevent the analytes on the sample surfaces 103 [illegible]

improve the mass spectrometric fragmentation, and/or to label the analytes or processed analytes to improve the mass spectrometric selectivity. After processing, intact analytes or mixtures of analyte subunits (e.g., protein subunits) can reside on the sample surfaces **103**, and the sample chip **105** and the dispenser chip **110** are separated from each other. The dispenser chip **110** may then be used to dispense liquids on other sample chips. The sample chip **105** may be analyzed immediately or stored and processed further at a later time.

FIG. 2(a) shows a cross-sectional view of a chip according to an embodiment of the invention. The illustrated chip includes a base **22** and sample structures **25(a), 25(b)** comprising pillars **20(a), 20(b)**. The base **22** and the pillars **20(a), 20(b)** may form an integral structure formed from the same material. Alternatively, the base **22** and the pillars **20(a), 20(b)** may be distinct and may be formed from different materials. Each pillar **20(a), 20(b)** may consist of a single material (e.g., silicon), or may include two or more sections of different material.

The base **22** of the chip may have any suitable characteristics. For instance, the base **22** of the chip can have any suitable lateral dimensions. For example, in some embodiments, the base **22** can have lateral dimensions less than about 2 square inches. In other embodiments, the base **22** can have lateral dimensions greater than 2 square inches. The non-sample surface of the base **22** may be generally planar. However, in some embodiments, the base **22** may have a non-planar surface. For example, the base **22** may have one or more troughs. The structures containing the sample surfaces and the pillars may be in the trough. Any suitable material may be used in the base **22**. Suitable materials include glass, silicon, or polymeric materials. Preferably, the base **22** comprises a micromachinable material such as silicon.

The pillars **20(a), 20(b)** may be oriented substantially perpendicular with respect to the base **22**. Each of the pillars **20(a), 20(b)** includes a sample surface **24(a), 24(b)** and side surfaces **18(a), 18(b)**. The side surfaces **18(a), 18(b)** of the pillars **20(a), 20(b)** can define respective sample surfaces **24(a), 24(b)** of the pillars **20(a), 20(b)**. The sample surfaces 24(a), 24(b) [illegible] sample surfaces **24(a), 24(b)** may have the same or different coatings or properties. Adjacent sample surfaces **24(a), 24(b)** are separated by a depression **27** that is formed by adjacent pillars 20(a), 20(b) and the non-sample surface **23**.

The pillars **20(a), 20(b)** may have any suitable geometry. For example, the cross-sections (e.g., along a radius or width) of the pillars may be circular or polygonal. Each of the pillars **20(a), 20(b)** may also be elongated. While the degree of elongation may vary, in some embodiments, the pillars **20(a), 20(b)** may have an aspect ratio of greater than about 0.25 or more (e.g., 0.25 to 40). In other embodiments, the aspect ratio of the pillars may be about 1.0 or more. The aspect ratio may be defined as the ratio of the height H of each pillar to the smallest width W of the pillar. Preferably, the height of each pillar may be greater than about 1 micron. For example, the height of each pillar may range from about 1 to 10 microns, or from about 10 to about 200 microns. Each pillar may have any suitable width including a width of less than about 0.5 mm (e.g., 100 microns or less).

Discrete volumes of liquid and can be present on the sample surfaces **24(a), 24(b)** of the pillars **20(a), 20(b),** respectively. The liquid samples may be deposited on the sample surfaces **24(a), 24(b)** in any suitable manner and with any suitable dispenser (not shown). The dispenser may include one or more passive valves within the fluid channels in the dispenser. Dispensers with passive valves are described in greater detail below.

The liquid samples may contain components (e.g., analytes, targets, capture agents) that are to be analyzed, reacted, or deposited on the sample surfaces **24(a), 24(b)**. Alternatively or additionally, the liquid samples may contain components that are to be deposited on the surfaces of the pillars **20(a), 20(b)** for subsequent analysis, assaying, or processing. For example, the liquid samples on the pillars **20(a), 20(b)** can comprise proteins. The proteins in the liquid samples may bind to the sample surfaces **24(a), 24(b)**. The proteins on the sample surfaces **24(a), 24(b)** can then be analyzed, processed, and/or subsequently assayed, or used as capture agents for capturing analytes.

The liquid samples on the adjacent sample surfaces **24(a), 24(b)** are separated from each other by the depression **27** between the adjacent structures. If, for example, a liquid sample flows off of the sample surface **24(a)**, the liquid sample flows into the depression **27** between the

[illegible] 18(a), 18(b) [illegible]

20(a), 20(b) may be rendered liquiphobic or may be inherently liquiphobic. For example, the side surfaces **18(a), 18(b)** may be coated with a hydrophobic material or may be

[illegible] 18(a), 18(b) [illegible]

pillars may also be coated with a material (e.g., alkane thiols or polyethylene glycol) resistant to analyte binding. The non-sample surface **23** may also be resistant to analyte binding or may be liquiphobic, or may consist partially or fully of the same material as the sample surfaces **24(a), 24(b)**.

In some embodiments, the pillars may have one or more channels that surround, wholly or in part, one or more pillars on the base. Examples of such channels are discussed in U.S. Patent Application No. 09/353,554 which is assigned to the same assignee as the present application and which is herein incorporated by reference in its entirety for all purposes. This U.S. Patent Application also discusses surface treatment processes and compound display processes that can be used in embodiments of the invention.

The top regions of the sample structures **25(a), 25(b)** may include one or more layers of material. For example, FIG. 2(b) shows a cross-sectional view of a chip with pillars **20(a), 20(b)** having a first layer **26** and a second layer **29** on the top surfaces **19(a), 19(b)** of the pillars **20(a), 20(b)**. In this example, the sample surfaces **24(a), 24(b)** of the structures **25(a), 25(b)** may correspond to the upper surface of the second layer **29**. In some embodiments, the top regions of the structures **25(a), 25(b)** may be inherently hydrophilic or rendered hydrophilic. As explained in further detail below, hydrophilic surfaces are less likely to adversely affect proteins that may be at the top regions of the structures **25(a), 25(b)**.

The first and the second layers **26, 29** may comprise any suitable material having any suitable thickness. The first and the second layers **26, 29** can comprise inorganic materials and may comprise at least one of a metal or an oxide such as a metal oxide. The selection of the material used in, for example, the second layer **29** (or for any other layer or at the top of the pillar) may depend on the molecules that are to be bound to the second layer **29**. For example, metals such as platinum, gold, and silver may be suitable for use with linking agents such as sulfur containing linking agents (e.g., alkanethiols or disulfide linking agents), while oxides such as silicon oxide or titanium oxide are suitable for use with linking agents such as silane-based linking agents. The

titanium and may be less than about 5 nanometers thick. The second layer **29** may comprise a noble metal such as gold and may be about 100 to about 200 nanometers

oxide or titanium oxide, while the second layer **29** may comprise a metal (e.g., noble metals) such as gold or silver. Although the example shown in FIG. 2(b) shows two layers of material on the top surfaces **19(a)**, **19(b)** of the pillars **20(a)**, **20(b)**, the top surfaces **19(a)**, **19(b)** may have more or less then two layers (e.g., one layer) on them. Moreover, although the first and the second layers **26**, **29** are described as having specific materials, it is understood that the first and the second layers **26**, **29** may have any suitable combination of materials.

The layers on the pillars may be deposited using any suitable process. For example, the previously described layers may be deposited using processes such as electron beam or thermal beam evaporation, chemical vapor deposition, sputtering, or any other technique known in the art.

In embodiments of the invention, an affinity structure may be on a pillar, alone or in combination with other layers. For example, the affinity structure may be on an oxide or metal layer on a pillar or may be on a pillar without an intervening layer. Preferably, the affinity structure comprises organic materials. In some embodiments, the affinity structure may consist of a single layer comprising molecules that are capable of binding to specific analytes (e.g., proteins). For instance, the affinity structure may comprise a single layer of capture agents that are bound to the surface of, for example, a metal or oxide layer on a pillar. The capture agents may comprise, for example, antibodies, antibody fragments, polypeptides, receptors, DNA strands, fragments, RNA strands or fragments, aptamers, etc. The capture agents can bind to components in a liquid medium through a covalent or a non-covalent mechanism. The affinity structure (and the elements of the affinity structure) can be used to increase the spacing between a top surface (e.g., a silicon surface) of a pillar and a protein that is attached to the top surface of the pillar. The spacing can decrease the likelihood that the attached protein might become deactivated by, for example contacting a solid surface of the sample structure.

In other embodiments, the affinity structure may comprise an organic thin film, affinity tags, adaptor molecules, and capture agents, alone or in any suitable

affinity structure. For example, the affinity structure may include three sublayers, each sublayer respectively comprising an organic thin film, affinity tags, and adaptor

The organic thin film, affinity tags, and adaptor molecules may have any suitable characteristics. An "organic thin film" is a normally a thin layer of organic molecules that is typically less than about 20 nanometers thick. Preferably, the organic thin film is in the form of a monolayer. A "monolayer" is a layer of molecules that is one molecule thick. In some embodiments, the molecules in the monolayer may be oriented perpendicular, or at an angle with respect to the surface to which the molecules are bound. The monolayer may resemble a "carpet" of molecules. The molecules in the monolayer may be relatively densely packed so that proteins that are above the monolayer do not contact the layer underneath the monolayer. Packing the molecules together in a monolayer decreases the likelihood that proteins above the monolayer will pass through the monolayer and contact a solid surface of the sample structure. An "affinity tag" is a functional moiety capable of directly or indirectly immobilizing a component such as a protein. The affinity tag may include a polypeptide that has a functional group that reacts with another functional group on a molecule in the organic thin film. Suitable affinity tags include avidin and streptavidin. An "adaptor" may be an entity that directly or indirectly links an affinity tag to a pillar. In some embodiments, an adaptor may provide an indirect or direct link between an affinity tag and a capture agent. Alternatively or additionally, the adaptor may provide an indirect or direct link between the pillar and, an affinity tag or a capture agent. The capture agent is preferably capable of capturing a protein from a liquid sample. In yet other embodiments, an adaptor may bind directly to a pillar or a layer on a pillar, and may be capable of binding to a component such as an analyte in a liquid sample. An example of a suitable adaptor is biotin. Other examples of organic thin films, affinity tags, adaptors, and capture agents are described in U.S. Patent Application nos. 09/115,455, 09/353,215, and 09/353,555, which are herein incorporated by reference in their entirety for all purposes, and are assigned to the same assignee as the present application. These U.S. Patent Applications describe various layered structures that can be on the pillars in embodiments of the invention.

The use of an affinity tag provides several advantages. For example, an affinity tag can confer enhanced bin[illegible]

The affinity struct[illegible] sublayers may be formed using any suitable process including, for ex[illegible]sorption, physisorption or chemoselective

materials, the pillars, or layers on the pillars by a covalent or a non-covalent bonding mechanism.

Examples of chip structures having affinity structures on the pillars are shown in FIGS. 3 and 4. FIG. 3 shows a cross-sectional view of a sample structure having an elevated sample surface. The sample structure includes a pillar **60**. An interlayer **61** including an oxide such as silicon oxide is at the top surface of the pillar **60**. The interlayer **61** may be used to bind the coating layer **62** to the pillar **60**. The coating layer **62** may include another oxide such as titanium oxide. An affinity structure **69** is on the coating layer **62**. The affinity structure **69** may include a monolayer **64** with organic molecules such as polylysine or polyethylene glycol. In some embodiments, the molecules in the monolayer **64** are linear molecules that may be oriented generally perpendicular to, or at an angle with, the surface the coating layer **62**. Each of the organic molecules in the monolayer **64** may have functional groups at both ends to allow the ends of the molecules to bind to other molecules. A set of molecules including a first adaptor molecule **65** such as biotin, an affinity tag **66** such as avidin or streptavidan, a second adaptor molecule **67** such as biotin, and a capture agent **68** such as an antibody are linked together. The set of molecules is bound to the monolayer **64**. In this example, the capture agent **68** is adapted to receive and capture an analyte in a liquid sample that is on the pillar **60**. For simplicity of illustration, only one set of molecules is shown in FIG. 3. However, it is understood that in embodiments of the invention, many such sets of molecules may be present on the monolayer **64**.

The embodiment shown in FIG. 3 has an affinity structure that has a number of sublayers. The affinity structures used in other embodiments of the invention may include more or less sublayers. For example, FIG. 4 shows a cross-sectional view of another sample structure having an affinity structure with fewer sublayers. The structure shown in FIG. 4 includes a pillar **70**. An interlayer **71** including a material such as silicon dioxide is at the top surface of the pillar **70**. A coating layer **72** including, for example, a metal oxide (e.g., titanium oxide) may be on the interlayer **71**. An affinity structure **78** may be on the coating layer **72**. The affinity structure **78** may include a monolayer **73** [illegible] may in turn bind an analyte **76** such as a protein to the affinity tag **74**.

The affinity structure components separate the sample surface from the top

contact with certain solid surfaces. The affinity structure may serve as a barrier between the pillar and any components in a liquid sample that are to be captured. This reduces the possibility that the top surface of the pillar may deactivate proteins in a liquid sample on the pillar. As shown in FIGS. 3 and 4, for example, the bound analyte **76** and the bound capture agent **68** are not in likely to contact a solid surface (e.g., the solid surfaces of the coating layers **62**, **72**). Consequently, the presence of the affinity structure **69**, **78** decreases the likelihood that contact sensitive molecules such as proteins will be adversely affected by contact with a solid surface. To further reduce this possibility, the materials of the affinity structure may contain materials that are less likely to inactivate proteins.

The pillars may be present in an array on a base of the chip. An example of an array of pillars is shown in FIG. 5. The pillar array may be regular or irregular. For example, the array may have even rows of pillars forming a regular array of pillars. The density of the pillars in the array may vary. For example, the density of the pillars may be about 25 pillars per square centimeter or greater (e.g., 10,000 or 100,000 per cm^2 or greater). Although the chips may have any suitable number of pillars, in some embodiments, the number of pillars per chip may be greater than 10, 100, or 1000. The pillar pitch (i.e., the center-to-center distance between adjacent pillars) may be 500 microns or less (e.g., 150 microns).

FIGS. 6(a)-6(b) show cross-sections of some pillar embodiments. FIG. 6(a) shows a pillar **24** that is integrally formed with respect to an underlying base **22**. In such embodiments, the base **22** may consist of the same material as the pillar **24**. FIG. 6(b) shows a pillar **24** that is on a base **22**. The pillar **24** may include, for example, a porous material such as a hydrogel material. In embodiments of the invention, all, part, or parts of the pillar may be similarly or differently porous (e.g., may have the same or different degree of porosity). For instance, different strata within a pillar may be porous and can have different properties. By using a porous material, liquid samples can pass into the porous material, and the pillar **24** can hold more liquid sample than would be possible if the pillar **24** was non-p[illegible]

Other suitable pillar shapes are shown in FIGS. 6(c) to 6(k). The embodiment shown in FIG. 6(i) includes a depression at the top portion of the pillar. [illegible]

FIGS. 6(j) and 6(k) show pillars with concave portions. In the embodiment shown in FIG. 6(j), each of the pillars **410, 420** has two non-concave portions **400, 402**, one portion **400** near the top and one portion **402** near the bottom. In this example, the side surface of each non-concave portion **400, 402** is substantially perpendicular to the top surface **406** of the pillar. A concave portion **404** lies between the two non-concave portions **400, 402**. Each pillar includes abrupt changes in geometry where the concave portion **404** begins and ends. The concave portions **400, 402** may be formed using, for example, a reactive ion etch process. FIG. 6(k) shows a pillar with a concave side surface that begins at the top surface of the pillar and ends at the bottom surface of the pillar.

Using pillars with concave portions and abrupt structural changes can be advantageous. For example, by providing concave portions to the pillars, more empty space is provided in the regions between adjacent pillars. For example, referring to FIG. 6(j), the volume V between the adjacent pillars **410, 420** can be used to contain any liquid sample that may flow off of the sample surfaces of the pillars **410, 420**. The volume V between adjacent pillars **410, 420** with concave portions is greater than the volume between adjacent pillars having substantially parallel side surfaces (compare, e.g., the pillars shown in FIG. 6(a)). Consequently, more space is provided to contain any liquids that may inadvertently flow off of the sample surfaces of the pillars. Moreover, the upper non-concave portion **400** of the pillar **410** shown in FIG. 6(j) has two structurally distinct edges E1, E2. As will be explained in further detail below, when pillars with abrupt structural changes (e.g., in FIG. 6(j)) are used, these structural changes can form two passive valves when used in conjunction with a dispenser with a cooperatively structured fluid channel. The two passive valves help to prevent a liquid sample from flowing down the sides of the pillars **410, 420**. Furthermore, if a liquid sample flows off of the sample surface on the pillar, the concave surface of the pillar can provide a path for the liquid sample to flow inwardly and away from an adjacent sample surface. This also reduces

[illegible] extends through both the base 290 and the pillars 299. A fluid **292** such as a gas may pass through the fluid passages **294** toward the sample surfaces on the pillars **299** to remove

be placed over the fluid passages **294** in the pillar **299** so that the apertures are over the sample surfaces. Gas may flow through the fluid passages **294** to carry processed samples **295** on the upper surfaces of the pillars **299** to an analytical device such as a mass spectrometer.

In a typical process of using the assembly shown in FIG. 6(l), liquids from a dispenser (not shown) may contact the sample surfaces on the pillars of a sample chip. The liquids may process substances on the sample surfaces on the pillars. For example, the liquids may comprise reagents that process proteins on the sample surfaces. After processing, the chip is separated from the dispenser, and the cover chip **291** is placed on the sample chip with the pillars **299**. The apertures of the cover chip **291** are respectively over the sample surfaces, and gas flows through fluid passages **294** that extend through the pillars **299**. The gas removes the processed substances from the sample surfaces and carries the processed substances through the apertures in the cover chip **291** and to an analysis device such as a mass spectrometer.

The sample chip shown in FIG. 6(l) can be used in other ways. For example, in other embodiments of the invention, liquids may also pass upwards through the fluid passages **294** and deposit on the sample surfaces of the sample chip (i.e., on the pillars). In yet other embodiments, the fluid passages **294** can be used to keep components at the sample surfaces hydrated. Hydrating gases or liquids (e.g., water) can pass through the fluid passages **294** to keep any components on the sample surfaces hydrated. For example, by keeping proteins on the sample surfaces hydrated, the proteins are less likely to denature. In some embodiments, the fluid passages **294** may be coupled to a sub-strata porous region of the pillar, useful, for example, to act as a liquid reservoir to supply liquid to the sample surface.

The pillars of the chip may be fabricated in any suitable manner and using any suitable material. For example, an embossing, etching or a molding process may be used to form the pillars on the base of the chip. For example, a silicon substrate can be patterned with photoresist where the top surfaces of the pillars are to be formed. An etching process such as a deep reactive ion etch may then be performed to etch deep

reactive ion etch process. If desired, the side surfaces of the formed pillars may be coated with material such as a hydrophobic material while the top surfaces of the pillars are

surfaces of the pillars. Processes for fabricating pillars are well known in the semiconductor and MEMS (microelectromechanical systems) industries.

Other embodiments of the invention are directed to dispenser assemblies. The dispenser assemblies according to embodiments of the invention may include a sample chip and a dispenser that can dispense one or more fluids on the sample surfaces of the chip. In some embodiments, a plurality of liquids may be supplied to the fluid channels in a dispenser. The liquids supplied to the different fluid channels may be the same or different and may contain the same or different components. For example, each of the liquids in respective fluid channels may include different analytes to be processed. In another example, the liquids in respective fluid channels may contain different capture agents to be coupled to the pillars of the sample chip. The dispenser may provide liquids to the sample surfaces in parallel.

The dispenser may have any suitable characteristics, and can be positioned above the sample chip when liquids are dispensed onto the sample chip. Pressure may be applied to the liquids to dispense the liquids. To control liquid flow, the dispenser may include passive or active valves. In some embodiments, the dispensers have at least one passive valve per fluid channel. In some embodiments, the dispensers may be in the form of a chip with a plurality of fluid channels. In these embodiments, each of the fluid channels can have an end that terminates at a bottom face of the dispenser chip. The dimensions of the fluid channels in the dispenser may vary. For example, a cross-sectional dimension of a fluid channel in the dispenser may be between about 1.0 to about 500 microns (e.g., about 1.0 to about 100 microns).

The dispensers used in embodiments of the invention may be made using any suitable process know in the art. For example, the dispenser may be made, for example, by a 3-D stereo lithography, mechanical drilling, ion etching, or a reactive ion etching process.

In some dispenser assembly embodiments, the sample structures of the chip may be cooperatively structured to fit into fluid channels in a dispenser. The sample structures and their corresponding sample [illegible] may be aligned with the fluid

[illegible] surfaces of the structures. For example, pressure (e.g., caused by pneumatic forces, electrophoretic or electrowetting forces) may be applied to a liquid in a fluid channel so [illegible]

embodiments, the distance between the sample surface and the liquid in a fluid channel may decrease until they contact each other. The chip and/or the dispenser may move toward each other to decrease the spacing between the sample surface and the liquid in the fluid channel. In these embodiments, pressure may or may not be applied to the liquid in the fluid channel.

The fluid channels in the dispenser may serve as reaction chambers (or interaction chambers) that can house respectively different interactions such as reactions or binding events. Each sample surface and the walls of a corresponding fluid channel may form a reaction chamber. In a typical assembly, each individual reaction chamber may house a different event (e.g., a different reaction or binding event). In other embodiments, the different reaction chambers may house the same types of events.

Illustratively, a dispenser may provide liquids to the sample surfaces of the chip structures. The liquids may contain molecules that may or may not interact with molecules bound to the sample surfaces of the chip. First, the sample structures containing the sample surfaces may be aligned with the fluid channels. After aligning, the sample surfaces may be inserted into or positioned proximate to the fluid channels. While the sample surfaces are in or proximate to the fluid channels, the liquids in the fluid channels of the dispenser flow and contact the sample surfaces. This allows the molecules bound to the sample surfaces and the molecules in the liquids to react or interact with each other in a nearly closed environment. The interactions or reactions can take place minimizing the exposure of the liquid samples on the sample surfaces to a gaseous environment such as air. Consequently, the likelihood that the liquid samples will evaporate is reduced. After a predetermined time has elapsed, the sample surfaces may be withdrawn from the fluid channels, and/or the chip and the dispenser may be separated from each other. The sample surfaces of the chip can then be rinsed. Products of the reactions or interactions may remain on the sample surfaces. The products at the sample surfaces may then be analyzed to determine, for example, if a reaction has taken place. Alternatively or additionally, the products on the sample surfaces may be further

Embodiments of the invention may be used to transfer liquids containing capture agents, analytes, etc. to sample surfaces of a chip without forming droplets. For

transferred from a dispenser to the chip. This minimizes the creation of liquid volumes with large surface-to-volume ratios. In embodiments of the invention, small volumes of liquids may be transferred to a chip and processed on the chip while minimizing alterations (e.g., protein denaturing) of components in the transferred liquids.

Some assembly embodiments may be described with reference to FIGS. 7 to 9. FIG. 7 shows a dispenser **110** and FIG. 8 shows a chip **105**. The chip **105** includes a plurality of pillars **101** on a base **105a**. Each pillar **101** has a top sample surface **103** and a side surface **104**. The sample surface **103** is elevated with respect to a non-sample surface of the base **105a**.

The dispenser **110** includes a body **111** having at least one fluid channel **112** defined in the body **111**. In this example, the fluid channels **112** are substantially vertical. As noted above, the fluid channels **112** may define reaction chambers that can house chemical or biological reactions or interactions. At least a portion of the fluid channels **112** may be oriented in a z direction with respect to an x-y plane formed by the body **111** of the dispenser **110**. In this example, the fluid channels **112** illustrated in FIG. 7 are vertical and have one end terminating at an upper surface of the body **111** and the other end terminating at a lower surface of the body **111**.

In other dispenser embodiments, the fluid channels **112** may have horizontal and vertical portions. For example, one end of a fluid channel may originate at an upper surface of the body and may pass horizontally across the upper surface of the body. At some predetermined point on the body, the orientation of the fluid channel changes from a horizontal orientation to a vertical orientation and terminates at a lower surface of the body of the dispenser. Moreover, although the number of fluid channels **112** in the dispenser is shown to be equal to the number of pillars **101** in the assembly shown in FIGS. 7 and 8, the number of fluid channels and the number of pillars of a chip may be different in other embodiments.

The walls defining the fluid channels **112**, as well as a bottom surface **113** of the dispenser **110** may be coated with various materials that influence the behavior of the liquid in the fluid channels **112**.

fluid channels **112** may be coated with a hydrophilic material. Proteins, for example, are less likely to denature if they come in contact with a hydrophilic surface than with a

The fluid channels **112** in the dispenser **110** may be cooperatively structured to receive the pillars **101**. For example, as shown in FIG. 8, the pillars **101** of the chip **105** may be insertable into the fluid channels **112** in the body of the dispenser **110**. In this regard, the axial cross-sectional area of each of the fluid channels **112** in the dispenser **110** may be greater than the axial cross-sectional area of the pillars **101**. When the pillars **101** are inserted into the fluid channels **112** in the dispenser **110**, the sample surfaces **103** of the pillars **101** may be within respective fluid channels **112**. The volumes defined by the fluid channels **112** and the top surfaces **103** of the pillars **101** may be reaction chambers where reactions can occur.

The chip **105** and the dispenser **110** may each have one or more alignment members so that they can be aligned with each other and the pillars can be aligned with the fluid channels. The alignment members may be alignment marks or alignment structures. Typical alignment structures may be, for example, a pin and a corresponding hole. For instance, the edges of the chip **105** may have one or more pins (not shown) that are longer than the pillars **101**. These pins may be inserted into corresponding holes (not shown) at the edges of the dispenser **110** to align the chip **105** and the dispenser **110** and consequently align the pillars **101** with the fluid channels **112**. The alignment members may be optical, mechanical, or magnetic. For example, in some embodiments, the alignment members may be high aspect ratio linear channels which permit light passage when, for example, the chip and the dispenser are operatively aligned. Alternatively, a magnetic region may induce a signal in a detector once, for example, the chip and the dispenser are operatively aligned

The dispenser assemblies may include one or more passive valves. A passive valve stops the flow of liquid inside or at the end of a capillary using a capillary pressure barrier that develops when the characteristics of the capillary or mini channel changes, such as when the capillary or channel cross-section changes abruptly, or when the materials of structures defining the fluid channels change abruptly. Passive valves are discussed in P. F. Man et al., "Microfabricated Capillary-Driven Stop Valve and Sample Injector," IEEE 11th Annual Int. MEMS Workshop, Santa Clara, California, Sept. 1998, pp. 210-220. These publications are herein incorporated by reference for all purposes. Passive valves are unlike active valves which completely close off a fluid channel with a

In an illustrative example of how an assembly with a passive valve can be used, the structures of a chip can be inserted into respective fluid channels in a dispenser. Each fluid channel can have one, two, or three or more passive valves. For instance, each fluid channel may have a passive valve that is formed by an abrupt structural change in the geometry of a fluid channel. For example, the walls of a fluid channel may form a step structure. When a liquid encounters the step structure at a predetermined pressure, the liquid stops flowing.

Passive valves can also be formed when the structures containing the sample surfaces are within or are positioned at the ends of the fluid channels. For example, a pillar may be inserted into a fluid channel so that there is a space between the side surfaces of the pillar that is in the fluid channel and the fluid channel walls around the pillar. The portion of the fluid channel where the pillar resides may have an annular configuration. As liquid flows towards the pillar, the geometry of the fluid channel changes from a cylindrical configuration to an annular configuration. At a predetermined pressure, the liquid stops flowing at this geometry change. Additional pressure is needed to cause the liquid to flow past this geometry change. Different pressures may be applied to initiate the flow of liquid past each of the passive valves in the fluid channel. For example, two different levels of pressure may be applied to a fluid in a fluid channel to move a liquid past two different passive valves.

In one specific example of an assembly with a dispenser using one or more passive valves, a chip including pillars is used with a dispenser containing a plurality of fluid channels. The pillars may be inserted into the fluid channels and the chip may be brought into contact with the dispenser. Before or after insertion, a first pressure is applied to the liquids in the fluid channels to push the fluid samples to, but not substantially past, the first passive valve. A second pressure is then applied to the fluid samples to push the samples past the first passive valve so that the liquids are in contact with the pillars. The samples do not pass the second passive valve, which is defined by the pillar and the channel walls. After the liquids in the fluid channels contact the sample surfaces, the pressure applied to the liquids is decreased. Then, the dispenser and the chip

[illegible] the sample surfaces. Withdrawing the pillars from the fluid channels may stop any events that may be occurring at the sample surfaces. Alternatively, reactions can still occur after the pillars are withdrawn from the fluid

channels and reactions can stop after a washing step is performed. After liquid samples are transferred to the sample surfaces, processes such as evaporation and the formation of an air-liquid interface will have little or no adverse effect on the deposited components in the liquid samples. Any residual solvent or material on the sample surface may be rinsed away leaving the desired components on the sample surfaces.

In other embodiments, the structures may be inserted into the fluid channels until contact is made with liquids within respective channels. In these embodiments, added pressure need not be applied to the fluids in the fluid channels to bring the fluids in contact with the sample surfaces of the structures.

The dispensers according to embodiments of the invention have a number of advantages. For instance, unlike conventional ring-pin dispensers, embodiments of the invention can deliver a large number of liquids to the sample surfaces in parallel. For example, in embodiments of the invention, 10,000 or more fluid channels can be used to dispense 10,000 liquid samples. In comparison, conventional ring-pin dispensers may have only about 30 ring pins per assembly. Also, unlike a capillary pin dispenser that can potentially physically touch a sample surface thus potentially damaging the dispenser and the sample surface, many of the described dispenser embodiments do not come in contact with the sample surface. Moreover, unlike many conventional dispensers, the assembly embodiments of the invention can reduce the likelihood of forming an air-liquid interface, since droplets are not formed when liquid is transferred from a dispenser to a chip. As the volume of a drop gets smaller, the surface to volume ratio of the drop gets larger leading to problematic interactions between the molecules in the liquid that are to be transferred to the sample surface and the air-liquid interface of the drop. In embodiments of the invention, droplets of liquid need not be formed, thus minimizing the formation of a liquid sample with a gas/liquid interface with a high surface to volume ratio.

Specific examples of assemblies using passive valves may be described with reference to FIGS. 10-14. Referring to FIGS. 10 and 11, a liquid **270** is placed in the fluid channel **112** in a dispenser **118**. A first dispenser portion **120(a)** may comprise a hydrophilic material and [illegible] 120(b) [illegible]

[illegible] dispenser **110** and the chip **100** are [illegible] with each other when the pillar **101** is inserted into the fluid channel **112** [illegible] after the pillar **101** is inserted into the fluid [illegible]

greater than atmospheric pressure. The liquid **270** flows to, but not past, a first passive valve **114** defined within the fluid channel **112**. The passive valve **114** may be formed by an abrupt change in the cross-sectional area of the fluid channel **112**. Alternatively or additionally, the passive valve **114** may be formed by an abrupt change in the material of the fluid channel walls (e.g., hydrophilic to hydrophobic). Regardless of the particular form that the passive valve **114** takes, the passive valve **114** prevents the liquid **270** from flowing out of the fluid channel **112** at the pressure P1.

Referring to FIG. 12, after the pillar **101** is inserted into the fluid channel **112**, a pressure P2 may be applied to the liquid **270**. The pressure P2 may be greater than the pressure P1. The applied pressure P2 causes the liquid **270** to flow past the first passive valve **114** and onto a material at the top surface **103** of the pillar **101** and to a second passive valve **115** defined by the top surface **103** of the pillar **101** and the surrounding walls of the fluid channel **112**.

Referring to FIG. 13, the abrupt change in geometry occurs at a fluid channel region **109** near the top surface 103 of the pillar **101**. In this example, this region **109** of the fluid channel **112** has an annular shape due to the presence of the pillar **101**. The liquid **270** reacts with the material on the top surface **103** of the pillar **101**. Alternatively, the liquid **270** and components in the liquid **270** may simply deposit on the top surface **103** of the pillar **101**.

After the liquid **270** is on the top surface **103** of the pillar **101**, the majority of the liquid **270** may be separated from the pillar **101**. For example, referring to FIG. 14, a pressure less than the pressure P2 (e.g., less than atmospheric pressure) is applied to the liquid **270** so that the bulk of the liquid **270** flows upward while leaving a portion of the liquid **270** on the pillar **101**. In other embodiments, the chip **105** and the dispenser **110** may be separated from each other to separate the bulk of the liquid **270** from the liquid deposited on the pillar **101**. The pillar **101** may be withdrawn from the fluid channel **112** and the bulk of the liquid **270** may be retained in the fluid channel **112** of the separated dispenser **110**. In some embodiments, separation of the pillar **101** from the fluid channel **112** may stop any interaction between the liquid and any material at the top surfaces of

from the chip, the top surface of the pillar 101 may be rinsed or flushed with another liquid. The rinsing or flushing step can stop any interactions between the liquid and any

material at the top surfaces of the pillar **101**, if the prior separation of the chip **105** and the dispenser **110** does not stop the interactions taking place.

FIG. 15 illustrates an assembly embodiment with a dispenser with a passive valve. The dispenser **110** has a fluid channel **112** having a first channel section **112a** communicating with a second channel section **112b**. The first channel section **112a** is wider than channel section **112b**. In this example, both the first channel section **112a** and the second channel section **112b** terminate in a shoulder **113** which forms a restriction between the first channel section **112a** and the second channel section **112b**. The restriction (or a preventative means for preventing the flow of liquid **270**) functions as a passive valve **114**. The internal walls of the channel **112** may have a hydrophobic surface **230**. The top surface **103** of the pillar **101** may be a hydrophilic surface **234**.

In the embodiment shown in FIG. 15, the liquid **270** may be deposited on the pillar **101** in the same or different manner as the processes described with reference to FIGS. 10-14. For instance, the pillar **101** may be inserted into or positioned at the end (e.g., exactly at the end of the fluid channel or just outside of the end of the fluid channel) of the fluid channel **112** of the dispenser **110**. The dispenser **110** may or may not contact the chip **105** during the process of depositing liquid onto the pillar **101**. When the flow of liquid **270** is stopped at the first passive valve, the liquid **270** may be at a pressure P_1. A second pressure P_2, which is greater than the first pressure P_1, is subsequently applied to the liquid **270** to force the liquid **270** through and past the first passive valve **114** until it contacts the hydrophilic surface **234** on the pillar **101** that lies within the fluid channel **112**. The upper portion of the pillar **101** and the surrounding fluid channel **112** may form a second restriction that forms a second passive valve. Alternatively, the hydrophilic surface **234** on top surface **103** of pillar **101** in combination with the hydrophobic surfaces **230** on the walls of second channel section **112b** and on side **104** of pillar **101** functions as the second passive valve. In both instances, the flow of the fluid **270** stops at the upper surface of the pillar **101**. The top surface of the chip base **105a** may also be a hydrophobic surface **230**. The bottom surface of the dispenser **110** may also be a hydrophobic surface **230**

and polymers terminating in hydrophilic groups (e.g., OH or COOH) may be used to form a hydrophilic surface **234**. The hydrophilic surface **234** on top of the pillars **101** may be

which is assigned to the same assignee as the present invention and is herein incorporated by reference in its entirety for all purposes.

FIG. 16 shows another assembly embodiment. This embodiment is similar to the embodiment shown in FIG. 15. However, in this example, the second channel section **112b** is on top of the first channel section **112a** and the liquid **270** passes through the second channel section **112b** before entering the first channel section **112a**. The walls of the channel **112** in this example have the hydrophilic surface **234**. A first pressure P_1 is applied to the liquid **270** to force the liquid **270** through the second channel section **112b** up to, but not past, a first passive valve **240**. In FIG. 16, the abrupt enlargement defines the first passive valve **240**. The abrupt enlargement is an instantaneous increase of the width of the fluid channel **112** defines a shoulder **113**. A second pressure P_2, which may be greater than the first pressure P_1, is subsequently applied to the liquid **270** to push the liquid **270** through and past the first passive valve **240** until contacting the hydrophilic surface **234** of the pillar **101**. The liquid **270** encounters a restriction defined by the pillar **101** when the pillar **101** is in the channel **112**. This restriction may function as a second passive valve. Alternatively or additionally, the hydrophilic surface **234** on top surface **103** and on internal wall of first channel section **112a** in combination with the hydrophobic surface **230** on the pillar chip **105**, including on the sides **104** of the pillar **101** may function as the second passive valve. The restriction prevents the flow of liquid **270** out of fluid channel **112** and onto the pillar chip **105**.

FIGS. 17(a) to 17(d) show cross sections of assembly embodiments including a chip with a pillar having a concave side surface. A sequence of steps that may be used to deposit a liquid sample onto a sample surface of a pillar may be described with reference to FIGS. 17(a) to 17(d).

FIG. 17(a) shows a pillar **322** on a base **320** of a chip. The pillar **322** includes a sample surface **322(a)** and a side including a concave portion **322(b)** between

sample flows past the step structure **303** of the dispenser **301**. The pressure P2 in this example is greater than the pressure P1. At the pressure P2, the liquid **340** may flow until it encounters the edge surfaces **322(c)**, **322(d)** of the upper non-concave portion. As shown in FIG. 17(b), the flow of the liquid **340** may stop at the upper edge **322(c)** of the pillar. The edge **322(c)** and a portion of the wall defining the fluid channel **341** may form a second passive valve that stops the liquid **340** from flowing past the edge **322(c)** at the pressure P2.

Alternatively or additionally, as shown in FIG. 17(c), the flow of the liquid **340** may stop at the bottom edge **322(d)** of the upper non-concave portion of the pillar **322** when the pressure P3 is applied to the liquid **340**. The edge **322(d)** and the surrounding wall may form a third passive valve that stops the liquid **340** from flowing past the edge **322(d)**. The pressure P3 may be greater than the pressures P1 and P2. Although pressure is applied to the liquid **340** in the examples shown in FIGS. 17(b) and 17(c), in other embodiments, a higher pressure need not be applied to the liquid **340** to bring the liquid **340** in contact with the sample surface **322(a)** of the pillar **322**. For instance, the pillar **322** and/or the dispenser **301** may move toward the other until they contact each other. Accordingly, in some embodiments, the sample surface and a liquid in a fluid channel can contact each other without applying additional pressure to the liquid **340**.

Advantageously, the pillar **322** shown in FIGS. 17(b) and 17(c) can, when in a fluid channel, form two passive valves proximate the upper portion of the pillar **322**. Having two passive valves instead of one to stop the flow of liquid at the top portion of the pillar **322** helps to ensure that a substantial amount of the liquid **340** does not flow down the sides of the pillar **322**. The flow of liquid **340** down the sides of the pillar **322** is further minimized and the likelihood that the liquid sample will flow to an adjacent sample surface is also minimized. This further reduces the likelihood of cross-contamination between samples on different sample surfaces.

Referring to FIG. 17(d), after the liquid **340** contacts the sample surface **322(a)** of the pillar **322**, a portion **327** of the liquid **340** may [illegible]

[illegible] pressure P4, which may be less than the pressures P2 and P3, may be applied to the liquid **340**. The lower pressure causes the liquid **340** to flow upward into the fluid channel **341**. [illegible]

other by moving the chip and/or the dispenser away from the other. If a portion **326** of the liquid sample does not deposit on the sample surface, it can flow down a side of the pillar **322** without flowing to a liquid sample **327** on an adjacent pillar **333**. Cross-contamination between samples on adjacent surfaces is thus minimized.

Additional details regarding dispenser assemblies and sample chips can be found in U.S. non-provisional application no. _____ entitled "Chips With Elevated Sample Surfaces" by Pierre F. Indermuhle et al. (Attorney Docket No. 020144-000810), which is herein incorporated by reference in its entirety for all purposes.

II. Analysis assemblies

After analytes or processed analytes are present at the sample surfaces of the sample chip, a microanalysis chip may be interfaced with the sample chip. The microanalysis chip may be used to perform functions including, but not limited to: transferring an analyte or analyte derivatives (e.g., mixture of subunits of an analyte) downstream of the sample surfaces of the sample chip; performing reactions at the sample surfaces of the sample chip (e.g., derivation reactions) or otherwise chemically affecting analytes; concentrating and/or purifying analytes or analyte derivatives in a fluid; performing infusion and/or performing chromatography on a fluid containing analytes or analyte derivatives; and delivering analytes or analyte derivatives to an analysis device such as a mass spectrometer.

FIG. 18 shows an analysis assembly comprising a microanalysis chip **200** over a chip assembly **100**. The microanalysis chip **200** can have an identifier such as a bar code **254**. Top views of the microanalysis chip **200** are shown in FIGS. 19 and 20. In FIG. 20, the fluid channels in the microanalysis chip **20** are embedded and are shown by dotted lines.

The chip assembly **100** comprises a chip holder **106** and a sample chip **105**. The sample chip **105** may be in a cooperatively structured depression in the chip holder **106** so that the upper surfaces of the chip holder **106** and the sample chip **105** are substantially coplanar. The sample chip **105** may be any of the above-described sample chips or chip assemblies. For example, the sample chip **105** may include sample structures with pillars **101** with side surfaces **103**. The sample surfaces **102** may be on the pillars **101**. The sample chip **100** may be in contact with a heat sink **104**

The microanalysis chip **200** may include a body **202** having a plurality of bottom openings that may be in the form of wells **225** defined within the body **202**. Specifically, one or more wells **225** may be at the channel bottoms of respective transfer-separation channels **226**. The transfer-separation channels **226** may be used to supply fluids to the sample surfaces **103** in the same of different manner as the above-described dispensers and dispenser chips. In some embodiments, the fluids passing through the transfer-separation channels **226** may be used to separate analytes or processed analytes from the sample surfaces **103**. The separated analytes may then be transferred downstream of the sample surfaces **103**. The wells **225** are coupled to a plurality of reservoirs **223** via a number of delivery channels **221**, a common delivery channel **240**, and a distribution network **224**. The distribution network **224** distributes fluids flowing through the common delivery channel **240** to the wells **225**. The distribution network **224** may include the transfer-separation channels **226**, and a number of valving and gating elements (not shown) to control the routing of fluids to the desired wells **225**.

The wells **225** may have any suitable geometry. For example, the wells **225** may be rectangular or cylindrical and may be cooperatively structured to receive the pillars **101** of the sample chip **105**. Each well **225** may also include one or more passive valves. The passive valves in the wells **225** may be the same or different than the passive valves in the fluid channels in the dispensers and the dispenser assemblies described above. Each well **225** may extend from the channel bottom of a transfer-separation channel **226** to a bottom surface of the microanalysis chip **200**. Accordingly, the fluids flowing in the transfer-separation channel **226** can be accessed through the bottom of the microanalysis chip **200**.

Each transfer-separation channel **226** may include a portion upstream of a well **225** at its channel bottom and a portion downstream of the well **225**. The downstream portion of the transfer-separation channels **226** may be in communication with a nozzle **227**. The nozzle **227** can discharge a fluid flowing in the transfer-separation channel **226** to an analysis device such as a mass spectrometer [illegible] discharge device.

In FIGS. 18 and 20, the wells **225**, the delivery channels **221**, the common delivery channel **240**, the distribution network **224**, and the transfer-separation channels **226** [illegible]

chip **200**. In other embodiments, the fluid channels can be at the top surface of the microanalysis chip **200**. For example, a plurality of transfer-separation channels **226** may be defined in the top surface of the microanalysis chip **200** so that they are open fluid channels.

The flow of fluids in the microanalysis chip **200** (or the dispenser chip **110** described above) may be accomplished by any means well known in the art. For example, pumping and valving elements (not shown) may control the flow of fluids through the various fluid channels in the analysis chip **200**. In some embodiments, electroosmotic pumping is used to control the flow of fluids in the analysis chip **200**. Electrodes (not shown) may be included the reservoirs **223** and at various points in the fluid channels in the analysis chip **200**. For clarity of illustration, many of the Figures do not show these electrodes. As known by those of ordinary skill in the art, the electrodes may be biased by applying predetermined potentials to them. The biased electrodes can then control the flow of fluids in the analysis chip **200**. The processes for incorporating such electrodes in a microfluidic device are well known in the art. Electroosmotic pumping techniques and other fluid flow control techniques are described in, for example, U.S. Patent Nos. 5,632,876; 5,750,015; 5,858,188 and 6,007,690. All of these U.S. patents are herein incorporated by reference for all purposes.

The flow of fluids in the fluid channels in the microanalysis chip may also be controlled by providing the fluid channels with a predetermined configuration or geometry. For example, when fluids are to be delivered at a similar rate, the fluid channels may have the same dimensions and may be parallel. In another example, if the fluids are to move faster in a particular fluid channel, the size of the fluid channel can be decreased relative to the size of other fluid channels. The flow of fluids in the microanalysis chip can be controlled in other ways. For example, external pressure or vacuum may be applied to the fluids in the fluid channels to control the flow of the fluids.

An illustrative use of the dispenser assembly can be described with reference to FIG. 18. After analytes are present at the sample surfaces **103** of the sample chip **105**, the sample chip **105** is subsequently interfaced with the microanalysis chip **200**

more procedures to process analytes at the sample surfaces **103**. For example, reagents may be delivered to the sample surfaces **103** to purify and concentrate intact analytes or

processed analytes (e.g., mixtures of peptide subunits) on the sample surfaces **103** prior to infusion or chromatographic separation of the analytes or processed analytes.

The sample chip **105** may be interfaced with the microanalysis chip **200** such that the pillars **101** of the sample chip **105** slidably pass into the wells **225** of the microanalysis chip **200**. The sample surfaces **103** on the pillars **101** are then in communication with fluids flowing through the transfer-separation channels **226**. One or more passive valves may be provided in the wells **225** to prevent fluids in the transfer-separation channels **226** from flowing out of them.

In some embodiments, reagents may be initially present in the reservoirs **223**. The reagents may flow from the reservoirs **223**, through the delivery channels **221**, to the common delivery channel **240**, to the distribution network **224**, to the transfer-separation channels **226**, and to the sample surfaces **103**. Analytes on the sample surfaces **103** can come into contact with reagents (or other fluids) flowing in the transfer-separation channels **226** so that analytes at the sample surfaces **103** can be processed. The reagents delivered to the sample surfaces **103** may be used to remove the analytes (subunit mixture(s) or intact analytes) from the sample surfaces. The reagents may also be used for subsequent fluid transfer, concentrating analytes, purifying analytes and/or performing a chromatography process. As may be desired, further reactions (e.g., derivatization, labeling) that may aide in the subsequent mass spectrometric analysis (e.g., to improve sensitivity and/or mass spectrometric fragmentation) may also occur prior to transfer or chromatography.

The analysis assembly may then be positioned in front of the sampling orifice of the mass spectrometer using any suitable translation stage system. The fluids in the transfer-separation channels **226** of the microanalysis chip **200** may be delivered to a sampling port of a mass spectrometer using the nozzles **227** in the microanalysis chip **200**. Depending on the characteristics of the mass spectrometer, the analysis may proceed in serial or parallel fashion. Intact analytes or subunit mixtures of the analytes may then be analyzed using the mass spectrometer.

Specific details [illegible]

[illegible] further detail. Each reservoir **223** may be a cavity that has a bottom surface **223a** and an open top **223b**. Reagents and other fluids may be supplied to the reservoirs **223** through [illegible]

basic), aqueous solutions of organic molecules, or solutions comprised of organic liquids. Each reservoir **223** may be coupled to a delivery channel **240** that may be coupled to a distribution network **224**.

Referring to FIG. 23 and 24, each transfer-separation channel **226** has a channel bottom **226a** that is interrupted by a well **225**. The well **225** has well walls **225a** and may be cooperatively arranged to receive a pillar **101** of the sample chip **105**. The sample chip **105** may be in a depression **106a** in the chip holder **106**, so that the upper surfaces of the chip holder **100** and the base of the sample chip **105** are substantially coplanar with each other.

When the pillar **101** is in the well **225**, the sample surface **103** on the pillar **101** may be substantially coplanar with the channel bottom **226a**. In other embodiments, the sample surface **103** may be above or below the channel bottom **226a**. If the sample surface **103** is below the channel bottom **226a**, the well **225** may include a passive valve. For example, the well **225** may include one or more passive valves that may be the same as, or different than, the passive valves described with reference to FIGS. 10 to 17(d).

The transfer-separation channels **226** may have any suitable characteristics. For example, exemplary transfer-separation channels **226** may have a cross-sectional dimension (e.g., a width) in the range of from about 0.1 um to about 500 microns, or about 0.1 to about 100 microns (e.g., about 1 to about 100 or about 500 microns). The cross-sectional profile of the transfer-separation channel **226** may be square, rectangular, trapezoidal, round, or any other shape. Although the transfer-separation channels **226** shown in many of the Figures are straight, other configurations are possible (e.g., curves, serpentine, etc.). The configuration of the fluid channels may be varied to maximize the density of fluid channels and/or enhance the effectiveness of the chip (e.g., separation efficiency). In some embodiments, the channel portions of the substrates may be enclosed by a cover. In an alternate embodiment, the transfer-separation channels **226** are partially covered or fully uncovered to provide direct assess to the fluids flowing in the transfer-separation channels **226**.

separate an analyte or a processed analyte before transferring it to an analysis device. For example, in some embodiments, after an analyte is processed at a sample surface **103**, the

concentration/chromatography zone **226z**. Additional analytes may subsequently be processed in a similar manner and may also be retained in the concentration/chromatography zone **226z**. Once the concentration/chromatography zone **226z** has the appropriate amount of analyte retained in it, an eluant fluid may pass through the concentration/chromatography zone **226z** to elute the analyte. The eluted analyte may then pass downstream of the sample surface **103** in the transfer-separation channel **226z** and to an analysis device such as a mass spectrometer. The analyte sample received at the mass spectrometer can have a high concentration of the processed analyte, thus making a subsequent analysis of the analyte less difficult. In other embodiments, the microanalysis chip need not have chromatography zones (see, e.g., FIGS. 25 and 26).

The concentration/chromatography zone may be composed of chromatographic packing material (e.g., beads, membrane, monolithic support, or chemically modified wall surface of the channel, or combinations there of). The concentration and purification of a fluid within the microanalysis chip may be accomplished by interaction with a stationary phase presented as particles in the channels or as a coating of the channel walls. The particles may have magnetic properties to allow for positioning of these particles within the channels. The stationary phase may be machined into the substrate of the channels using photolithography or other suitable means. In instances where chromatography is desirable, any number of modes may be utilized such as electrophoretic or liquid chromatography. Those skilled in the art will understand that chromatography includes, for example, affinity, ion, hydrophobic, reversed-phase as well as electrophoretic chromatographies (e.g., electrophoresis, isotachophoresis, electrochromatography, isoelectric focusing). The employed chromatography may be also multidimensional and not limited the number of modes or their ordering. For example, multimode separations such as electrophoresis followed reversed-phase separation may be employed. Any number of standard concentration schemes (e.g., solid-phase extraction, isotachophoresis) may be performed prior to or instead of chromatography and/or infusion of samples (e.g. subunit mixtures from analytes or intact analyte molecules).

terminates in a nozzle 227. The [illegible] 227 can be used to control the discharge of fluid from the microanalysis chip 200. [illegible] ample, the discharge portion **226c** is [illegible] channel 226

The nozzle **227** may be of any suitable type and may have any suitable characteristics. For example, the nozzles used in embodiments of the invention may have an inside diameter of about 0.1 to 100 microns and an outside diameter of about 1 to 500 microns. Exemplary nozzles that can be used include those described in PCT publication number WO 00/06388 entitled "Micro-Mechanically Produced Nozzle for Producing Reproducible Droplets," and PCT publication number WO 00/15321 entitled "Integrated Monolithic Microfabricated Electrospray and Liquid Chromatrography System and Method". These publications are herein incorporated herein by reference for all purposes.

The nozzles may be fabricated in any suitable manner. For instance, the nozzles may be formed using a deep reactive ion etching (DRIE) process. Alternatively, the nozzles may be made from capillary tubes that are then inserted into fluid channels in the analysis chip. The capillary tubes may made of, for example, glasses, polymers, metals, or composite materials.

FIGS. 25 to 26 show analysis assembly embodiments with a seal member **253** between a microanalysis chip **200** and a sample chip **105**. The seal member **253** prevents fluid leakage from around the pillars **101** out of the wells **125** and the transfer-separation channels **226**. For example, the seal member **253** prevents liquid from flowing out of wells **125** and onto the sample chip **105**. Assemblies comprising a seal member **253** may be employed with or without the passive valves. In some embodiments, the seal member **253** may include a soft, elastomeric polymeric material (e.g., polydimethylsiloxane). Also, the configuration of the seal member **253** may vary. In one embodiment, the seal member **253** may be a ring that seals the periphery region between a microanalysis chip and a chip assembly. In another embodiment, the seal member **253** may be in the form of a perforated layer. The pillars of a sample chip can extend through the perforations in the perforated layer.

FIGS. 27 to 29 show an analysis assembly comprising a microanalysis chip **200** and a sample chip **105**. In this example, the microanalysis chip **200** includes a lid **230** that supports the nozzles **227**. The lid **230** may have nozzles **227**. Each nozzle **227** defines a discharge aperture **201**.

the discharge nozzles are present. Each analysis chip includes a nozzle **227** and a discharge aperture **201**. The embodiments shown in FIGS. 30-33 also include a well

discharge channel portions **226c** in the transfer-separation channels **226**. The discharge channel portions **226c** are substantially perpendicular to the other portions of the transfer-separation channels **226**.

III. Analytical systems

The dispenser assemblies and analytical assemblies may be used in an analytical system. The analytical system can include an analysis device such as a mass spectrometer. In other embodiments, the analysis device may be an optical analysis device or an electrical analysis device.

FIG. 35 shows an exemplary analytical system. The analytical system **300** includes multiple chip assemblies **100** that may be on a support element **301**. Each chip assembly **100** may include one or more sample chips. Each sample chip may include sample structures comprising pillars and samples surfaces. The support element **301** may have depressions that are structured so that a chip assembly **100** may be secured to the support element **301**.

The support elements **301** containing the chip assemblies **100** may be stacked in a first stacking device **303**. The first stacking device **303** may then unload chip assemblies **100** in a sequential manner onto a conveyor system **304** that allows movement of the chip assemblies **100** to multiple stations. A first station may be a reagent dispensing station **305** where a dispensing chip **110** is interfaced with a sample chip of a corresponding chip assembly **100**. The dispensing chip **110** may dispense reagents onto the sample surfaces of a sample chip. The dispensed reagents may include, for example, substances that can cleave proteins into peptide subunits. Next, the chip assemblies **100** are transported to a second station.

At the second station, a second stacking device **306** may contain a stack of microanalysis chips **200**. A microanalysis chip **200** may be interfaced with a sample chip of a chip assembly **100** at the second station to form an analysis assembly **307**. As

[illegible] may then be presented to a mass spectrometer **309**. A movable stage device **308** may move the analysis assembly **307** in an x-, y-, and/or z- direction. In some embodiments, [illegible] in the analysis assembly **307** [illegible] stage device **308**.

Processed analytes can be delivered from the nozzles of the analysis assembly **307** to the sample orifice. The mass spectrometer **309** can then be used to analyze the received analytes. Analyzed sample chips and their associated chip holders may then be stored in a third stacking device **310** after analyzing.

The system embodiments of the invention provide a number of advantages. For example, the system shown in FIG. 35 can be used to continuously process a number of sample chips, each having a plurality of analytes. In comparison to 2-D gel processes, for instance, embodiments of the invention can be used to process and analyze significantly more analytes in less time and at reduced cost.

The terms and expressions which have been employed herein are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding equivalents of the features shown and described, or portions thereof, it being recognized that various modifications are possible within the scope of the invention claimed. Moreover, any one or more features of any embodiment of the invention may be combined with any one or more other features of any other embodiment of the invention, without departing from the scope of the invention. For example, any feature of the sample structures, pillars or the passive valves described with reference to FIGS. 2 to 17 can be incorporated into any of the analysis assemblies, microanalysis chips, or systems shown in FIGS. 18 to 35 without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. A microanalysis chip comprising a body defining at least one transfer-separation channel including a channel bottom having a bottom opening, the transfer-separation channel terminating in a discharge aperture.

2. The microanalysis chip of claim 1 further comprising a seal member positioned against a bottom surface of the body.

3. The microanalysis chip of claim 1 wherein the bottom opening forms a well.

4. The microanalysis chip of claim 1 wherein the bottom opening comprises a passive valve.

5. The microanalysis chip of claim 1 further comprising a reservoir in the body and a reagent fluid in the reservoir.

6. The microanalysis chip of claim 1 comprising a plurality of the transfer-separation channels.

7. The microanalysis chip of claim 1 wherein the bottom opening is cooperatively structured to receive a pillar of a sample chip.

8. The microanalysis chip of claim 1 wherein the body comprises one of silicon, glass, or polymeric materials.

9. The microanalysis chip of claim 1 further comprising a reservoir and a reagent adapted to process proteins contained in the reservoir.

10. The microanalysis chip of claim 1 further comprising a fluid distribution network.

12. The microanalysis chip of claim 1 wherein the at least one transfer-separation channel is positioned within the body.

13. The microanalysis chip of claim 1 further comprising a chromatography/retention zone downstream of the bottom opening.

14. The microanalysis chip of claim 1 further comprising a lid and a nozzle, wherein the lid has a nozzle.

15. A method for chemically affecting a sample comprising:

providing a microanalysis chip including a body having a transfer-separation channel with a channel bottom having a bottom opening;

inserting a pillar into the bottom opening such that a sample supported by the pillar communicates with the transfer-separation channel; and

passing a reagent fluid into the transfer-separation channel in order for the reagent fluid to come in contact with the sample to chemically affect the sample.

16. The method of claim 15 wherein the pillar is on a base.

17. The method of claim 16 further comprising:

sealing a region between the microanalysis chip and the base with a seal member.

18. A dispenser assembly comprising:

a dispenser chip including a dispenser body including a vertical channel; and

a sample chip having a base and a sample structure, the sample structure comprising a pillar and a sample surface, wherein the vertical channel of the dispenser chip is cooperatively structured to receive the pillar.

19. The dispenser assembly of claim 19 further comprising:

a seal member between the dispenser body and the base of the sample chip.

a plurality of transfer-separation channels in the body, each transfer-separation channel [illegible] bottom with a bottom opening, and

having a portion upstream of the bottom opening and a portion downstream of bottom opening, and wherein each transfer-separation channel terminates at one of the discharge apertures.

21. The microfluidic chip of claim 20 further comprising:
a plurality of reservoirs coupled to the transfer-separation channels.

22. The microfluidic chip of claim 20 further comprising:
a plurality of reservoirs; and
a plurality of delivery channels upstream of the plurality of transfer-separation channels.

23. The microfluidic chip of claim 20 further comprising:
a plurality of nozzles, each nozzle containing one of the discharge apertures.

24. The microfluidic chip of claim 20 further comprising:
a lid having a plurality of nozzles, each nozzle containing one of the discharge apertures.

25. The microfluidic chip of claim 20 wherein the bottom opening includes a passive valve.

26. The microfluidic chip of claim 20 wherein each transfer-separation channel comprises a concentration/chromatography zone in the portion of the transfer-separation channel downstream of the opening.

27. The microfluidic chip of claim 26 wherein the discharge apertures are at a top surface of the microfluidic chip.

28. A microfluidic assembly comprising:
a microfluidic chip comprising (i) a body having a bottom surface, (ii) a

opening, and having a portion upstream of the bottom opening and a portion downstream of bottom opening, and wherein each transfer-separation channel terminates at one of the

a sample chip comprising a base including a non-sample surface and a plurality of sample structures, each sample structure including a sample surface.

29. The microfluidic assembly of claim 28 wherein the sample surfaces are elevated with respect to the non-sample surface.

30. The microfluidic assembly of claim 28 wherein each sample structure comprises a pillar, wherein the sample surface is on the pillar.

31. The microfluidic assembly of claim 28 wherein the bottom opening comprises a passive valve.

32. The microfluidic assembly of claim 28 further comprising:
a seal between the microfluidic chip and the sample chip.

33. The microfluidic assembly of claim 28 wherein the microfluidic chip further comprises:
a plurality of reservoirs, each reservoir containing a reagent;
a plurality of delivery channels coupled to the plurality of reservoirs; and
a distribution network of fluid channels coupled to the plurality of delivery channels.

34. A method of processing an analyte, the method comprising:
processing an analyte on a sample surface on an sample chip;
transferring the processed analyte through a transfer-separation downstream of the sample surface, wherein the transfer-separation channel is in a microfluidic chip above the sample chip; and
analyzing the processed analyte downstream of the sample surface.

35. The method of claim 34 wherein analyzing the processed sample comprises analyzing the processed sample using mass spectrometry.

inserting the sample surface into a fluid channel in a dispenser chip, wherein the sample surface is on a pillar.

depositing a liquid sample on the sample surface using the dispenser chip; and

binding an analyte in the liquid sample to the sample surface.

37. The method of claim 34 wherein processing comprises:

dispensing a reagent on the sample surface; and

cleaving the analyte into subunits.

38. A microfluidic chip comprising:

a body having a bottom surface; and

a plurality of vertical channels in the body, wherein each opening is cooperatively structured to receive a pillar of a sample chip.

39. The microfluidic chip of claim 38 wherein the body further comprises:

a plurality of horizontal delivery channels in communication with the plurality of vertical channels.

40. The microfluidic chip of claim 38 wherein the body further comprises:

a plurality of reservoirs upstream of the plurality of vertical fluid channels.

41. The microfluidic chip of claim 38 the body comprises silicon, glass, or polymeric materials.

42. The microfluidic chip of claim 38 wherein surfaces of the body forming each vertical channel are hydrophobic.

43. The microfluidic chip of claim 38 wherein surfaces of the body forming each vertical channel are hydrophilic.

44. A method of processing analytes, the method comprising:

depositing a plurality of liquid samples on the sample surfaces while the sample surfaces are in the

binding analytes from the plurality of liquid samples to the sample surfaces;

withdrawing the sample surfaces from the vertical fluid channels;

inserting the plurality of sample surfaces into a plurality of openings in a microanalysis chip so that the plurality of sample surfaces are in communication with a plurality of transfer-separation channels in the microanalysis chip; and

processing the analytes using reagents flowing through the transfer-separation channels while the analytes are bound to the sample surfaces.

45. The method of claim 44 further comprising:

discharging the processed analytes from the microanalysis chip using a plurality of nozzles in the microfluidic chip.

46. The method of claim 44 further comprising:

transferring the processed analytes to a mass spectrometer.

47. The method of claim 44 wherein the analytes are proteins, DNA, or RNA.

48. The method of claim 44 wherein processing includes at least one of derivatizing, cleaving, or unfolding the analyte.

49. The method of claim 44 wherein each vertical fluid channel comprises a passive valve.

50. The method of claim 44 wherein each pillar has an aspect ratio greater than about 1.

51. The method of claim 44 further comprising:

performing a chromatography process on the processed analytes.

52. The method of claim 44 further comprising:

the transfer-separation channels.

53. A system for analyzing analytes, the system comprising:

an analysis assembly comprising (i) a microanalysis chip comprising a body comprising at least one transfer-separation channel defined by a channel bottom having a bottom opening, the transfer-separation channel terminating in a discharge aperture, and (ii) a sample chip having a plurality of sample surfaces; and

an analysis device adapted to receive an analyte from the discharge aperture.

54. The system of claim 53 wherein the analysis device is a mass spectrometer.

55. The system of claim 53 wherein the sample surfaces are on pillars.

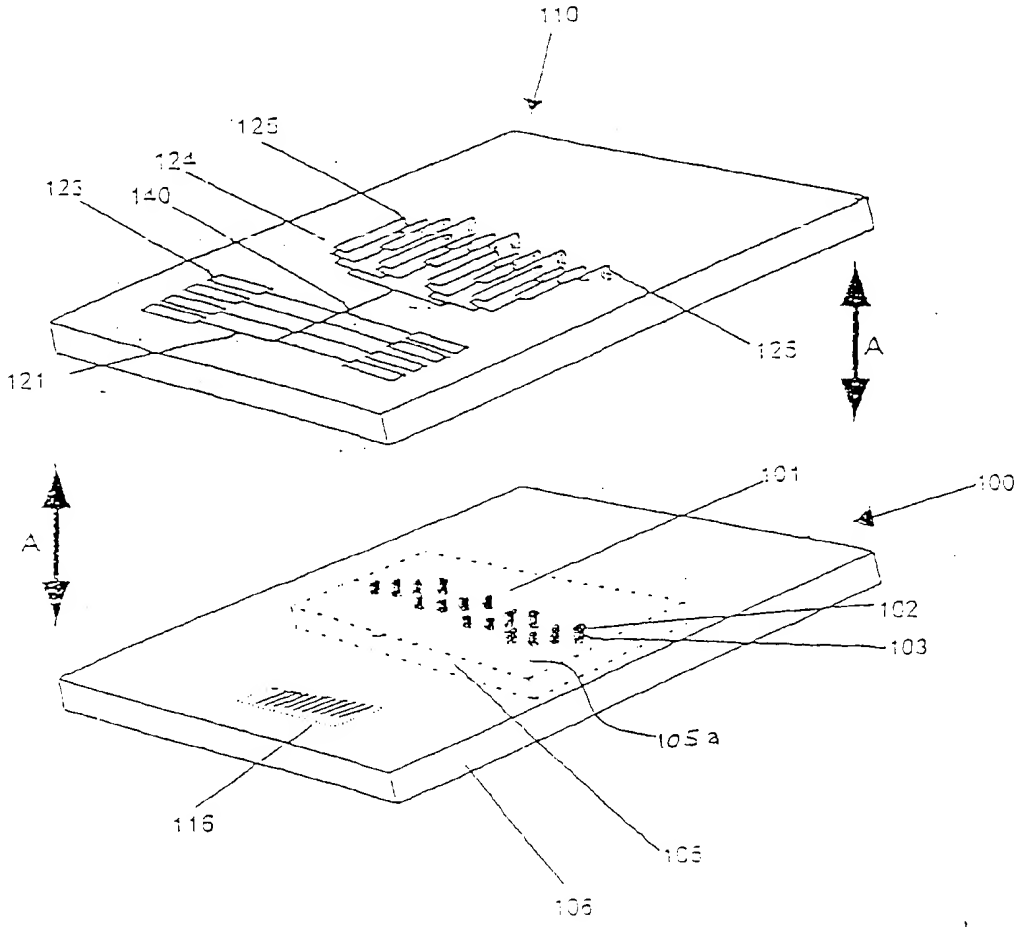


FIG. 1

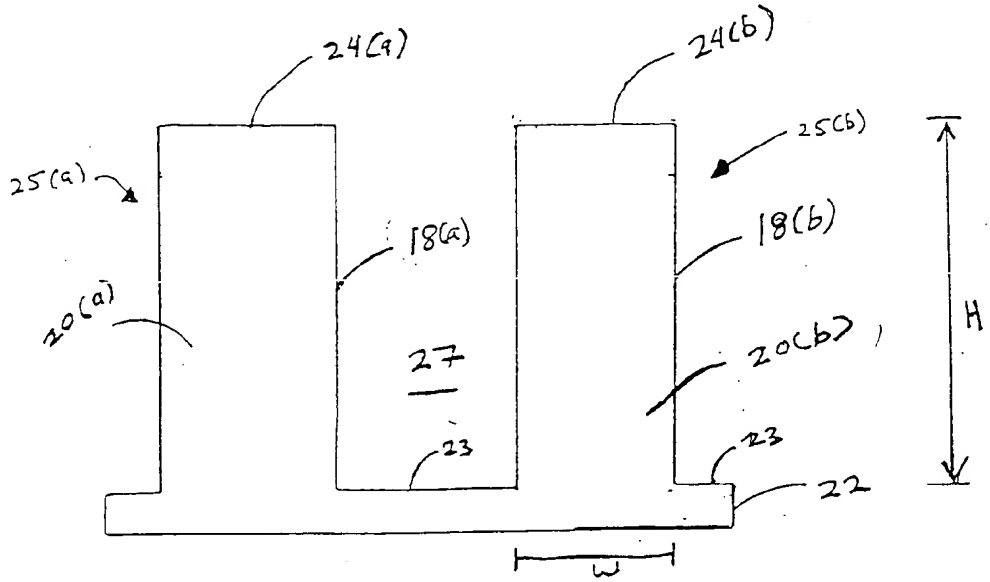

24(a)
24(b)
25(a)
25(b)
18(a)
18(b)
20(a)
20(b)
27
23
23
22
H
W

FIG 2(a)

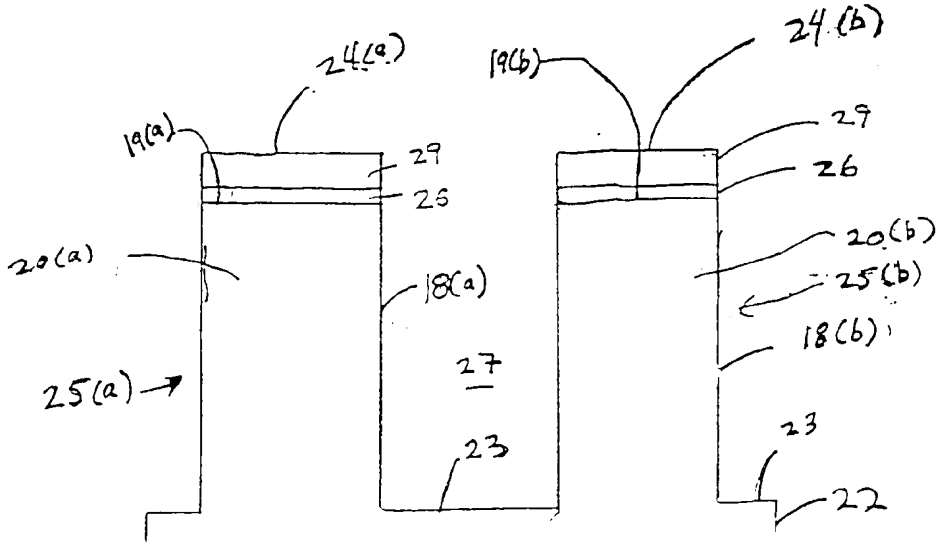

24(a)
24(b)
19(a)
19(b)
29
29
26
26
20(a)
20(b)
18(a)
25(b)
18(b)
25(a)
27
23
23
22

FIG. 2(b)

FIG. 3

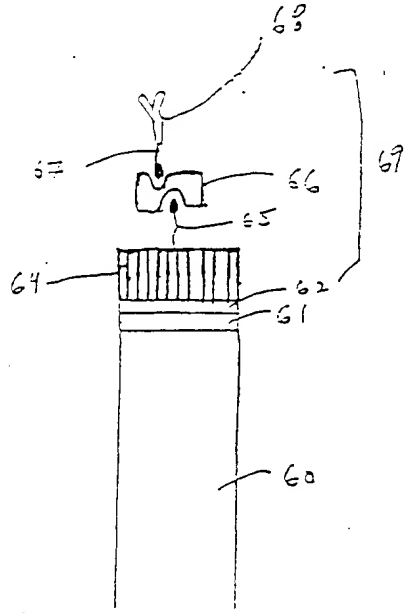
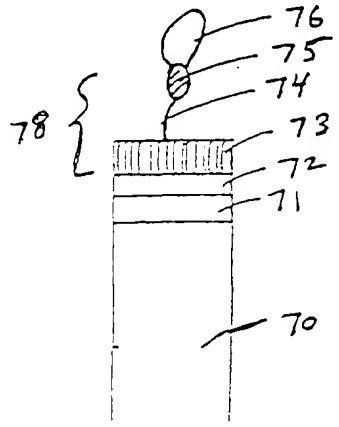


FIG. 4



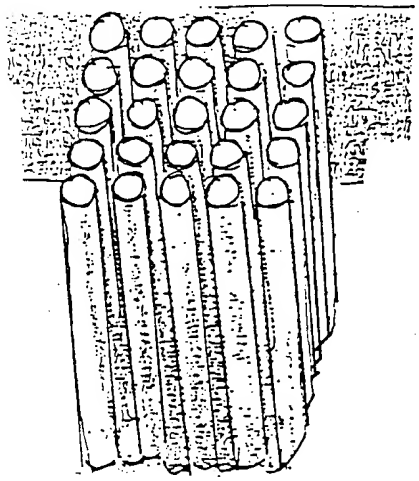


FIG. 5

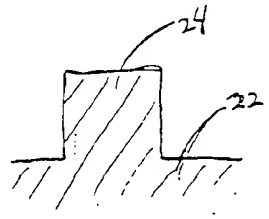


FIG. 6(a)

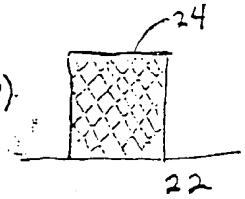


FIG. 6(b)

FIG. 6(c)

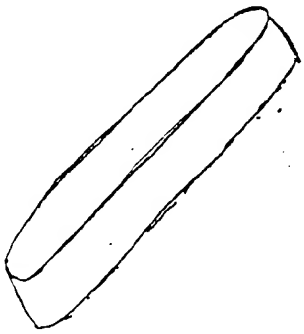


FIG. 6(d)

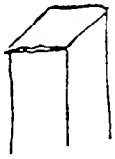


FIG. 6(e)

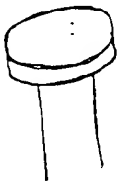


FIG. 6(f)

FIG. 6(i)

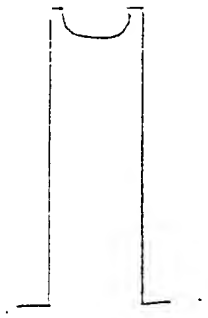


FIG. 6(j)

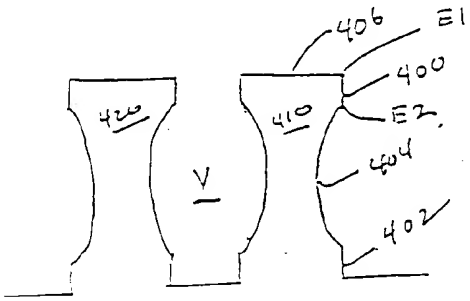


FIG. 6(k)

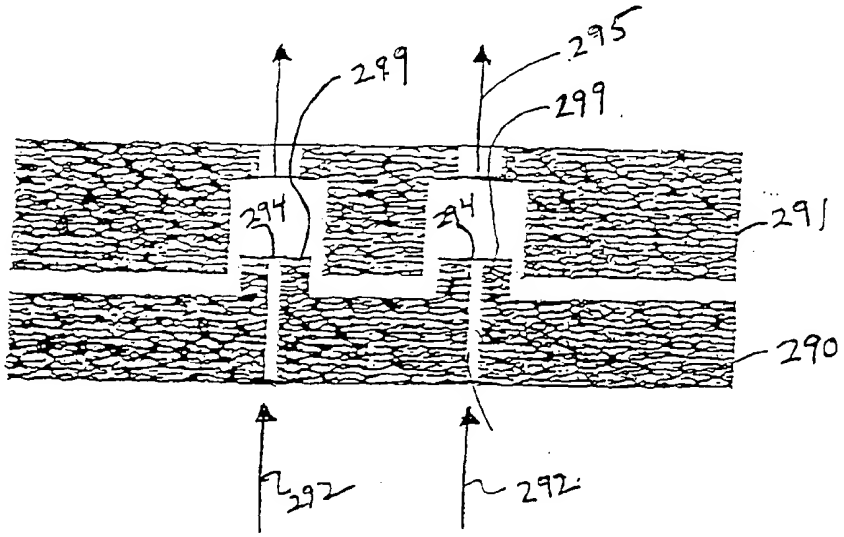
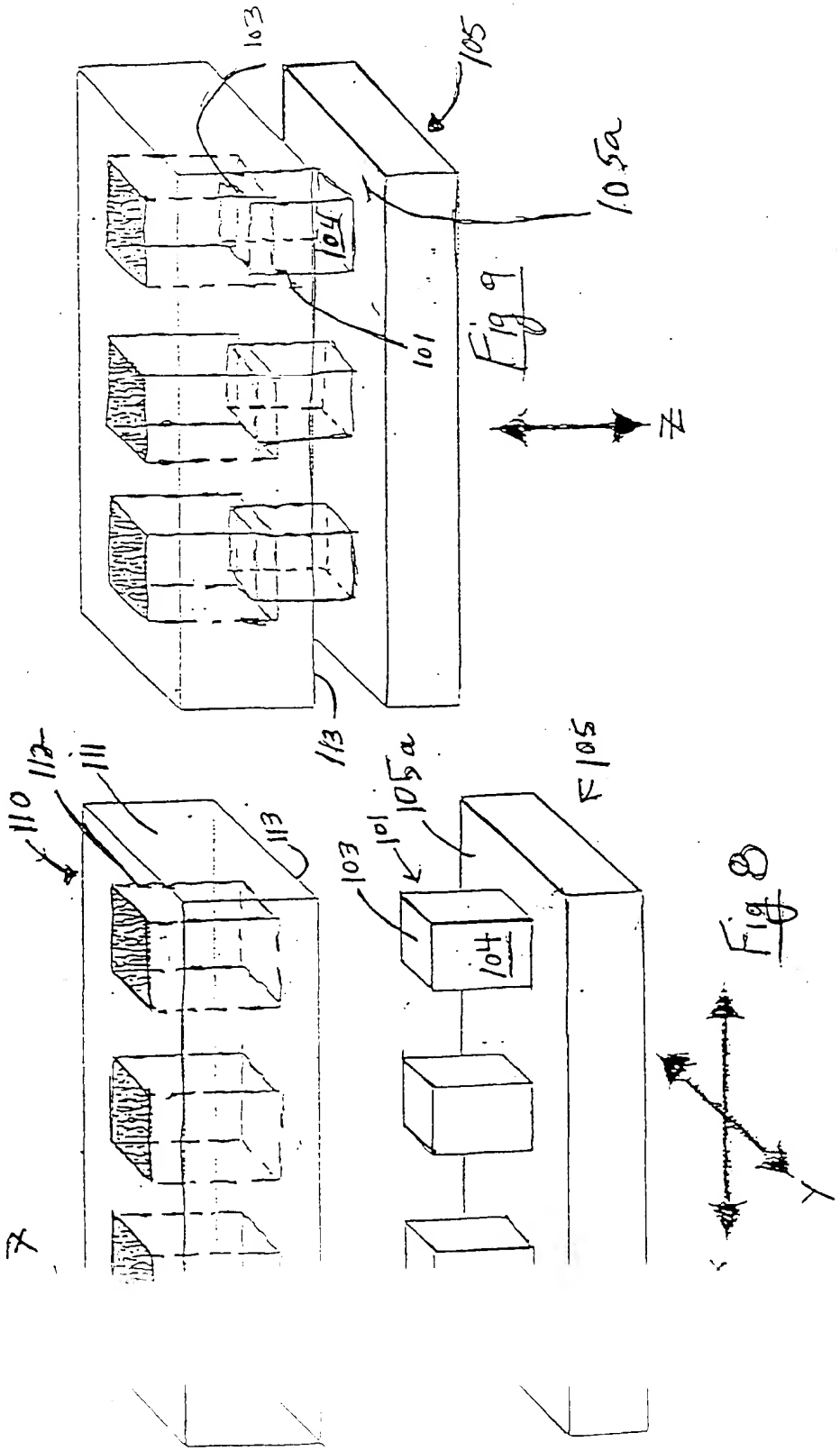


FIG. 6(2)


103
105
105a
101
104
Z
Fig 9
111
112
110
113
Y
Fig 8

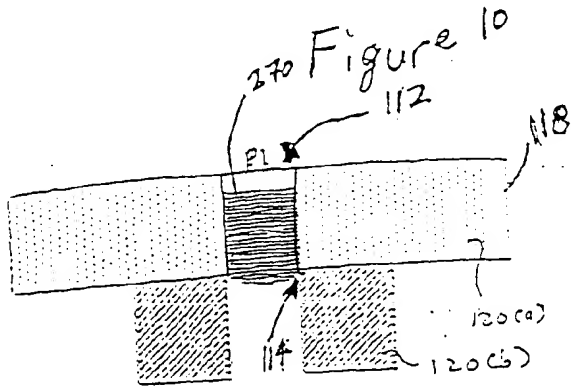


Figure 10

P1 > Patmospheric

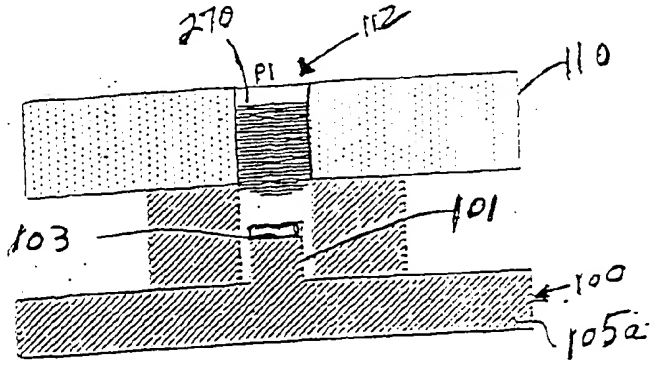


Figure 11

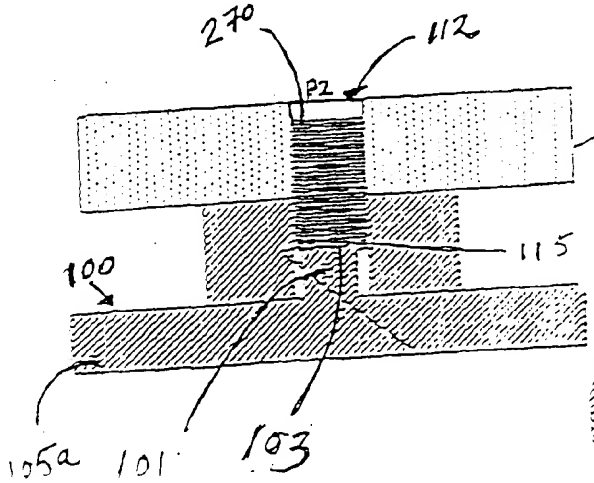


Figure 12

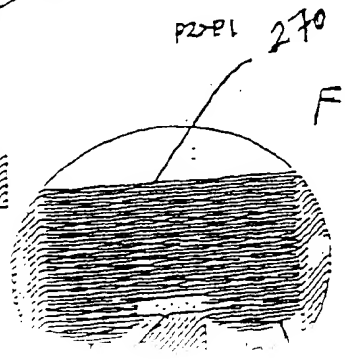


Figure 13

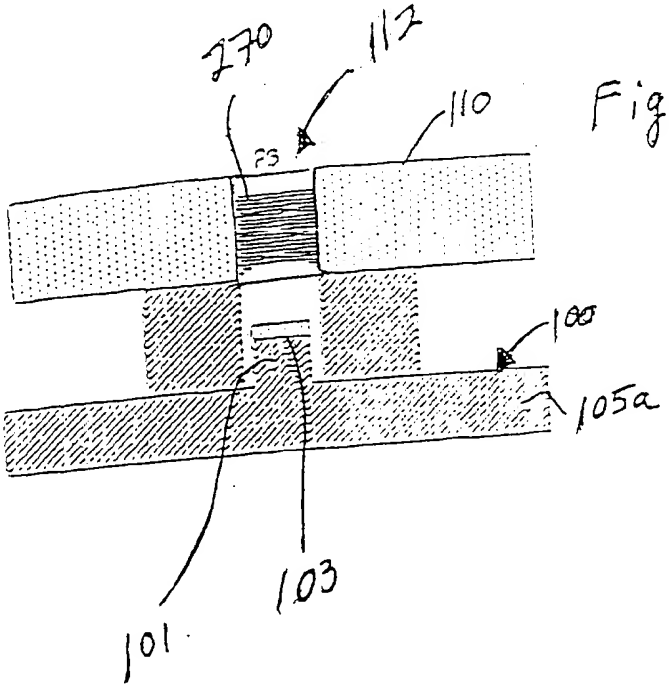
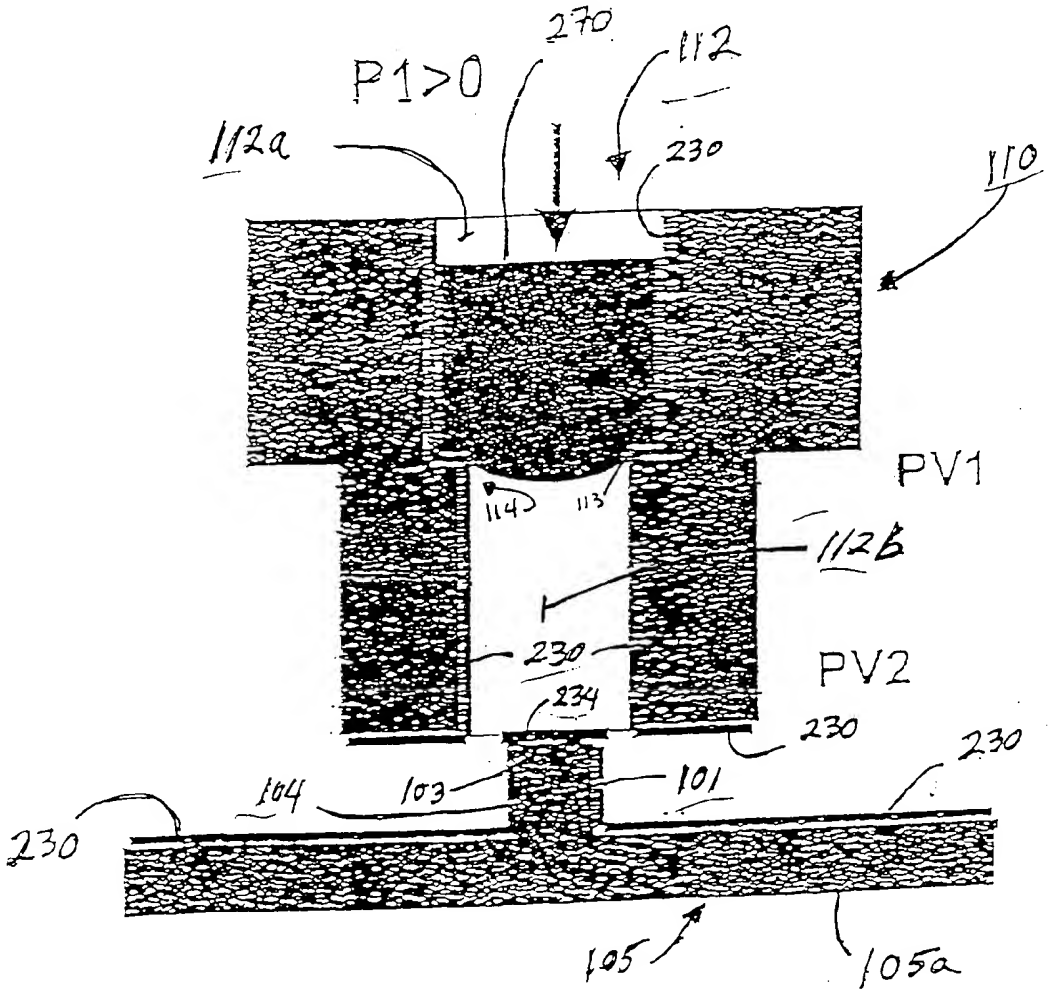
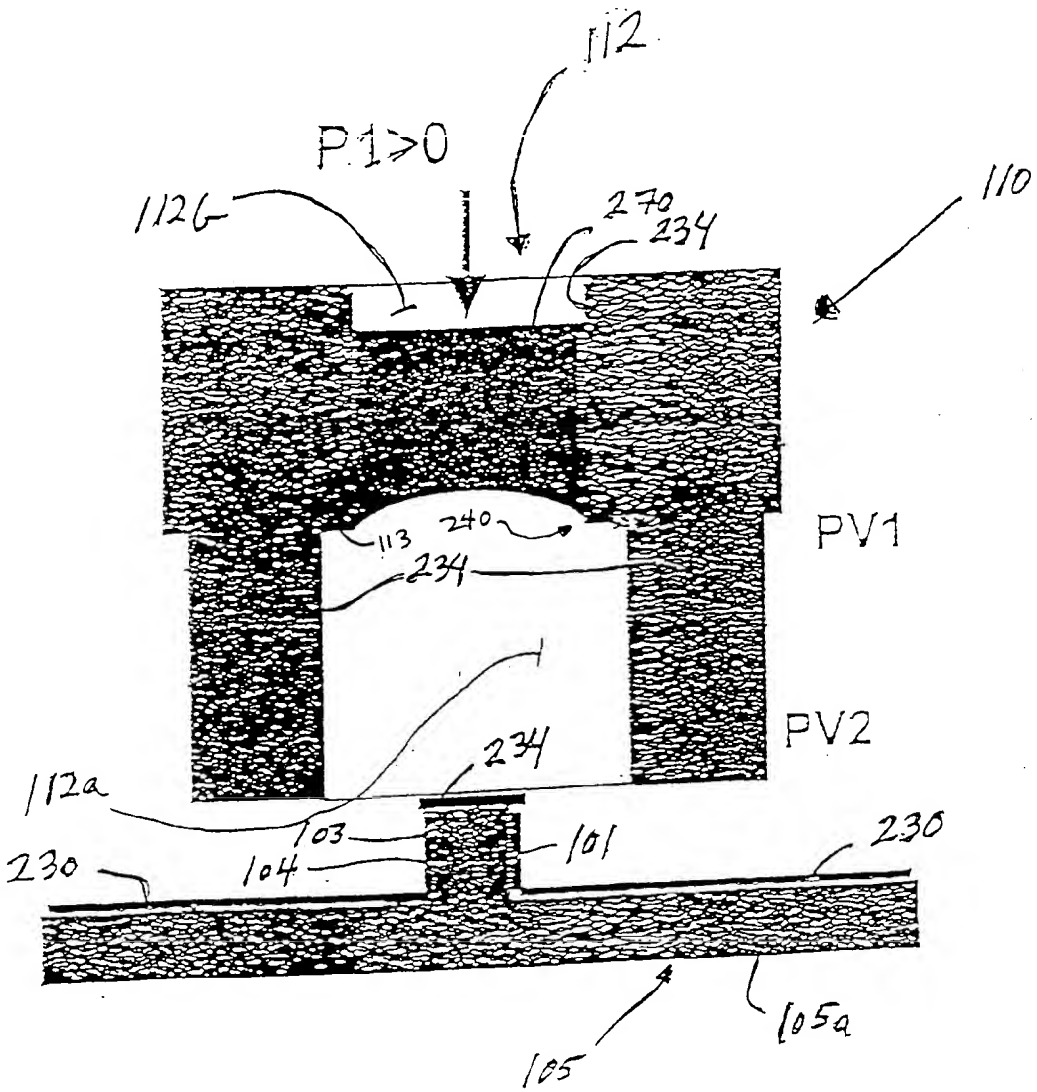


Figure 14

P3 < P ATMOSPHERIC


270
112
P1>0
112a
230
110
PV1
114
113
112B
230
PV2
234
230
230
230
104
103
101
230
105
105a


112
P1>0
112b
270
234
110
PV1
113
240
234
PV2
234
112a
103
101
230
230
104
105a
105

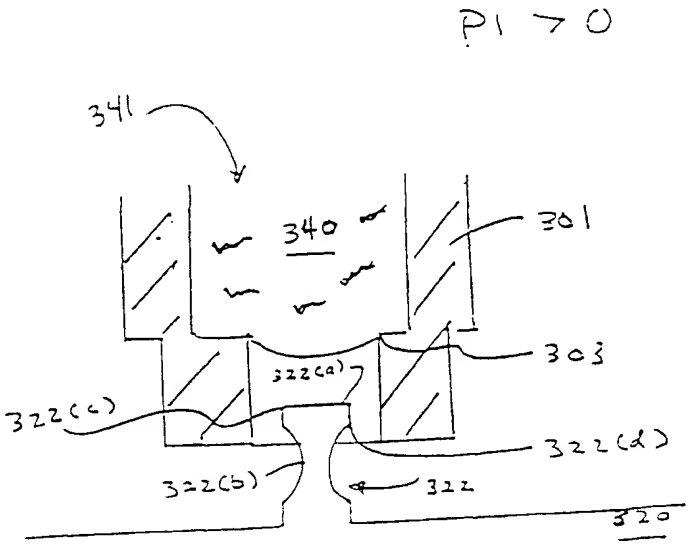
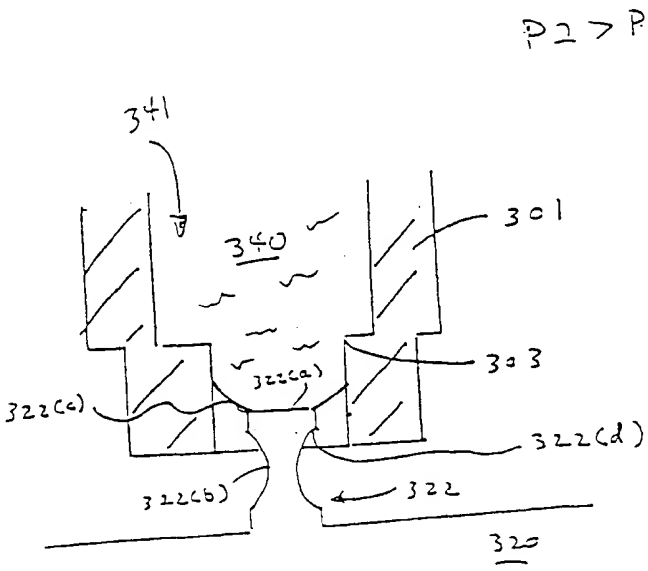
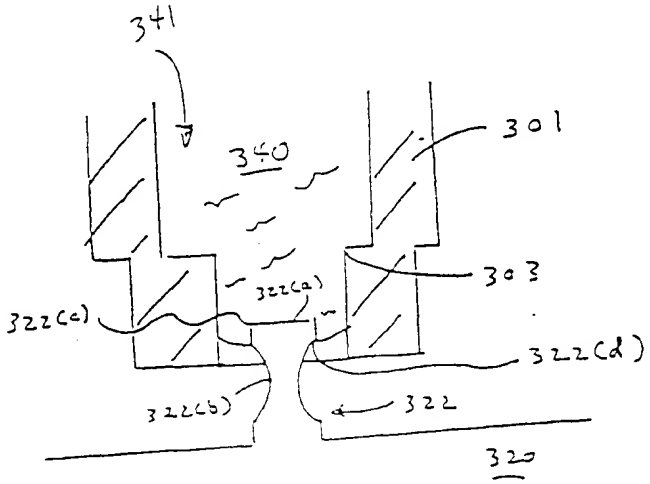
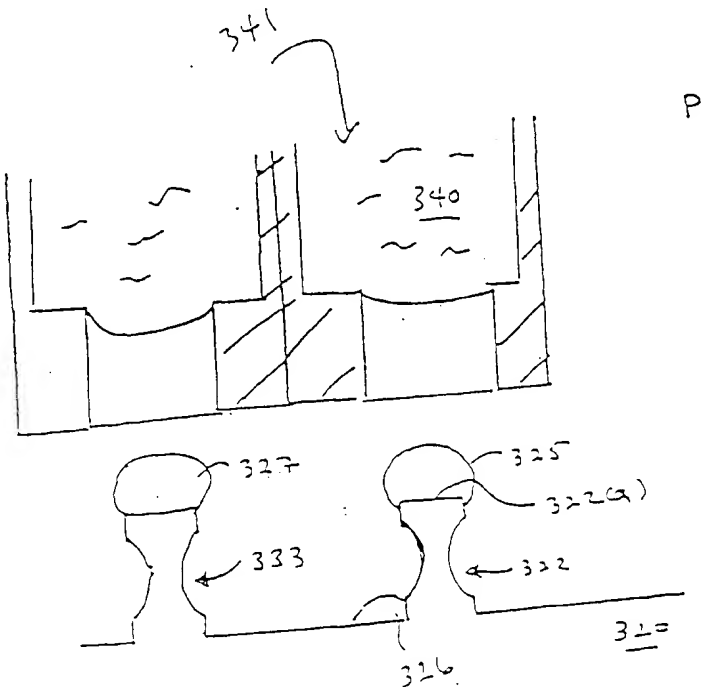


FIG. 3(a)


P2 > P1
341
340
301
303
322(a)
322(c)
322(d)
322(b)
322
320

P37 P2





P4 < P2

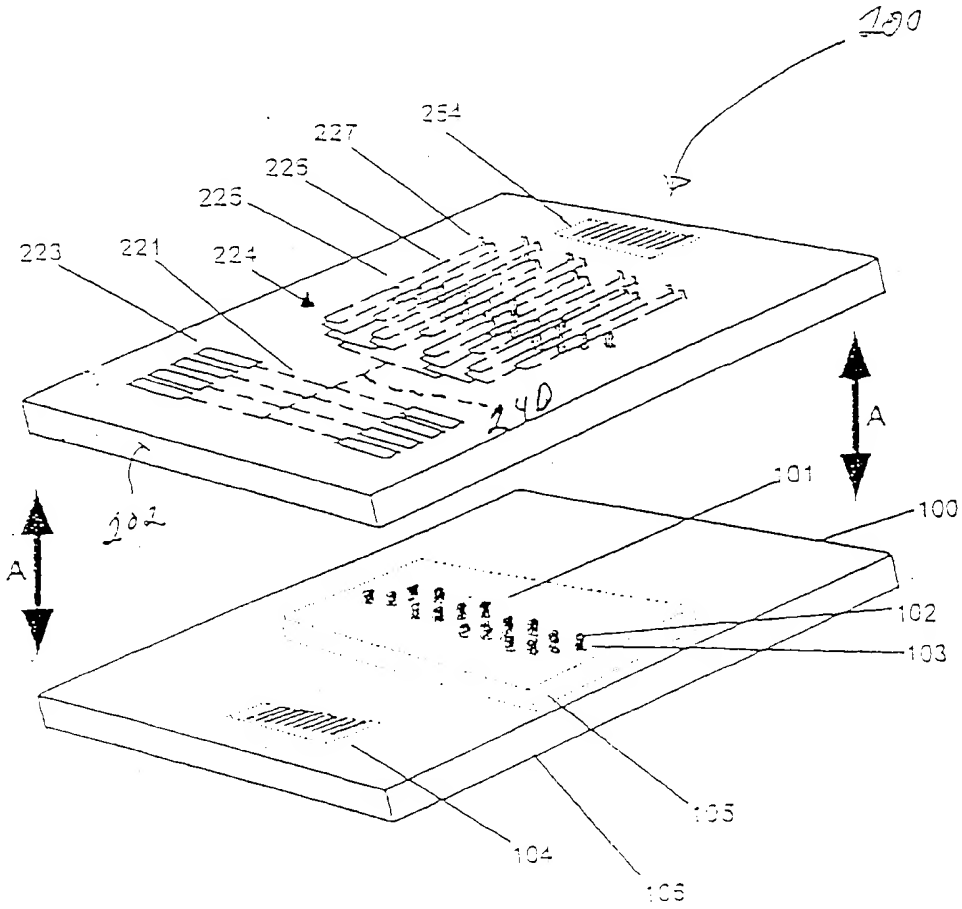

120
254
227
226
225
223
221
224
240
A
101
100
102
A
102
103
105
104
106

FIG. 19

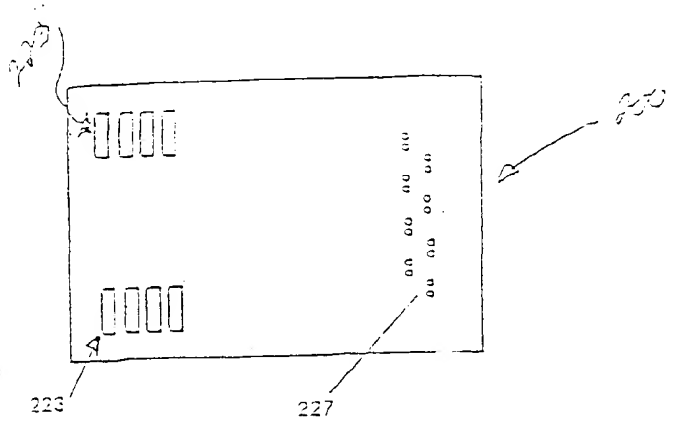


FIG. 20

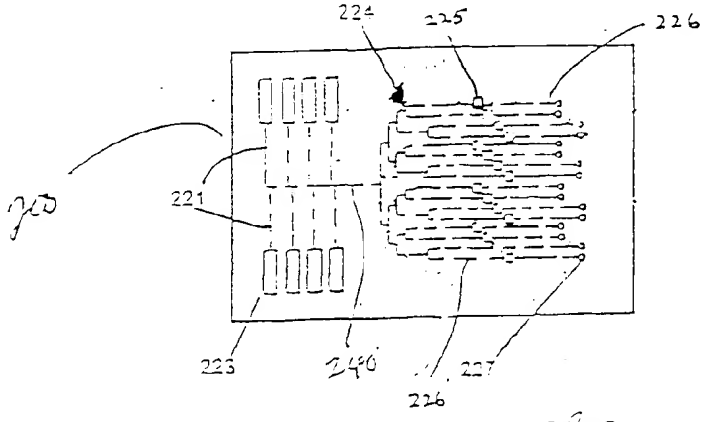


FIG. 21

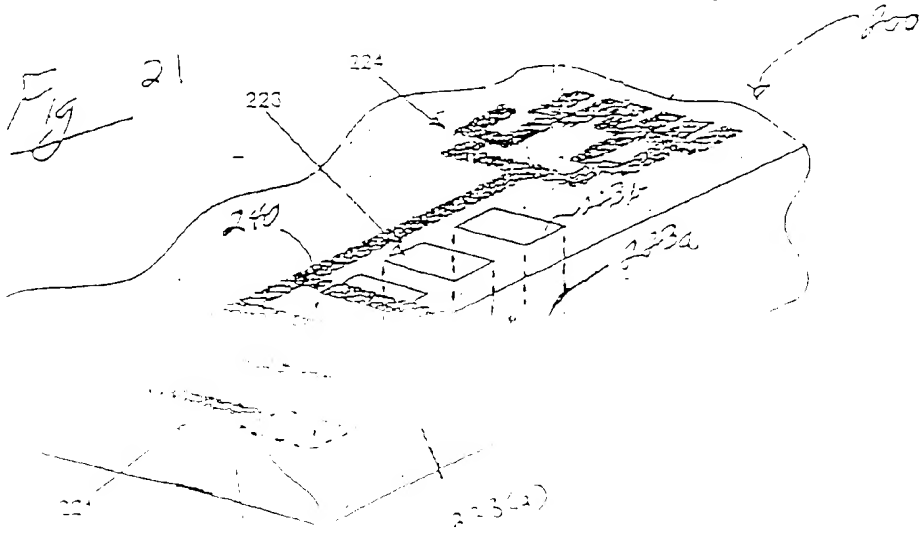


Fig 22A

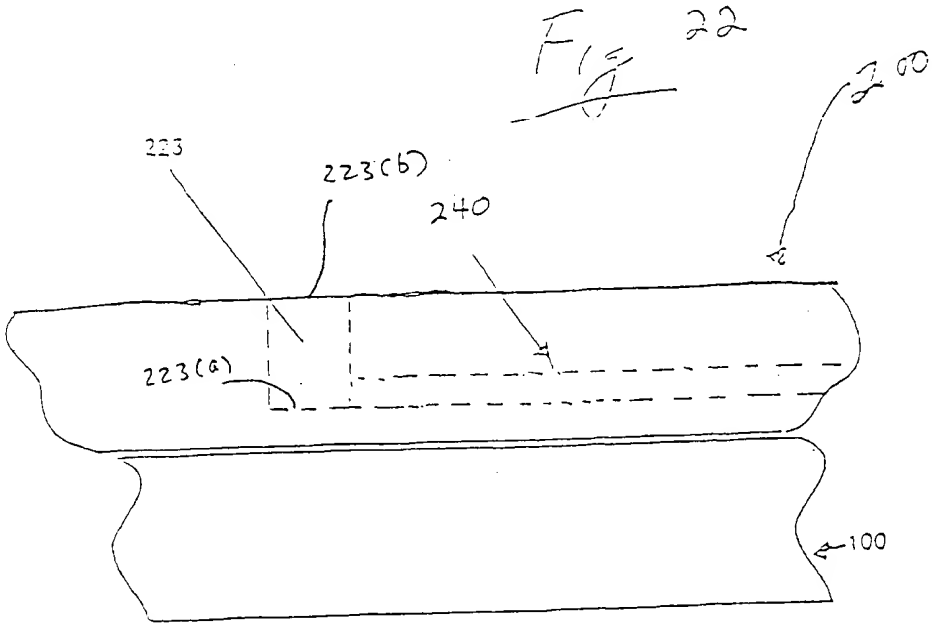


FIG. 23

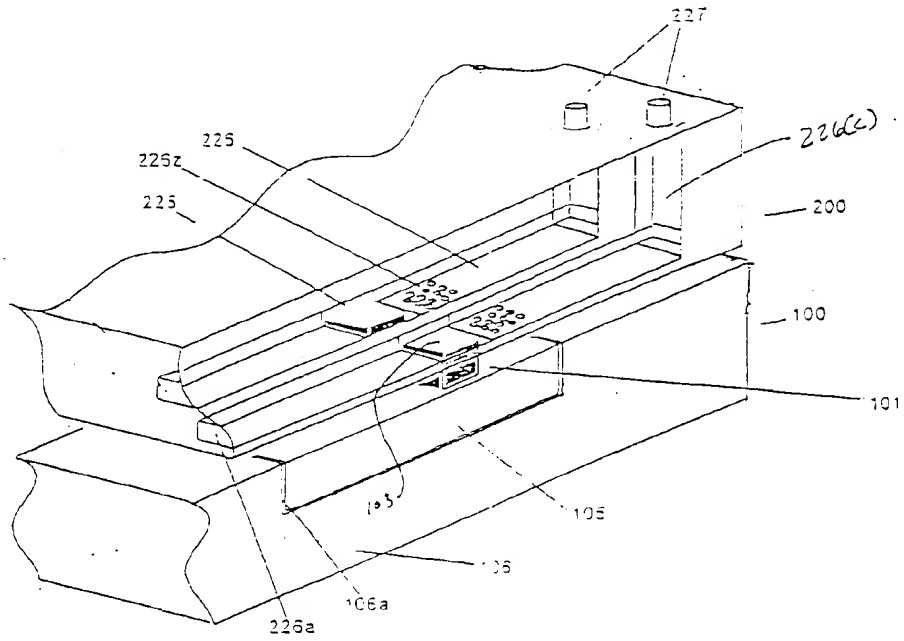

227
225
225z
225
226(c)
200
100
101
105
106a
226a

FIG. 24

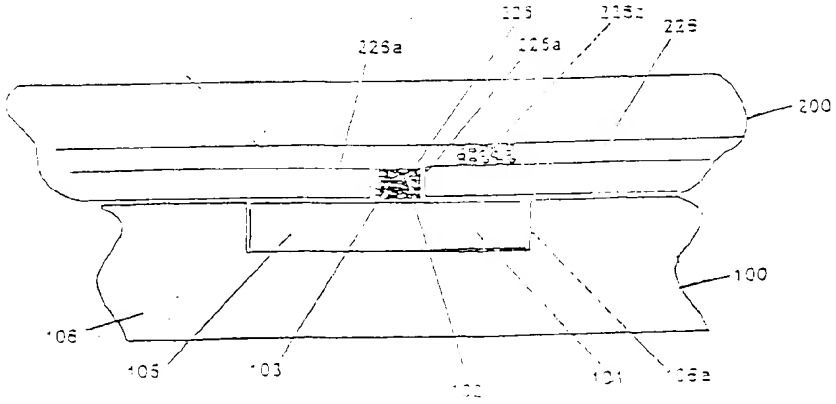

225
225z
226
225a
226a
200
100
106
105
103
101
106a

FIG. 25

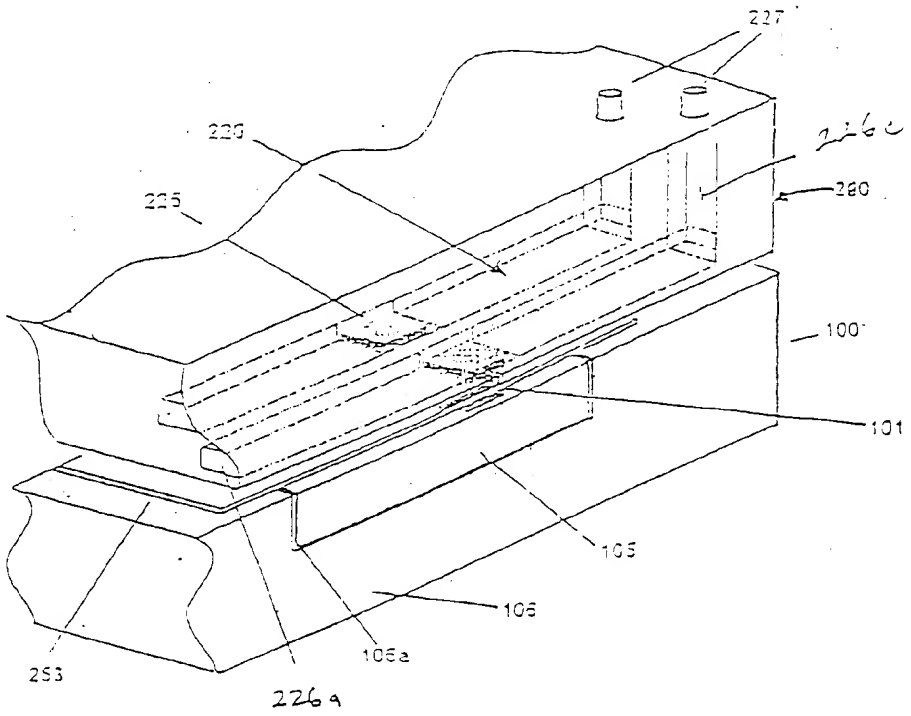

227
220
226c
225
280
100
101
105
106
106a
253
226a

FIG. 26

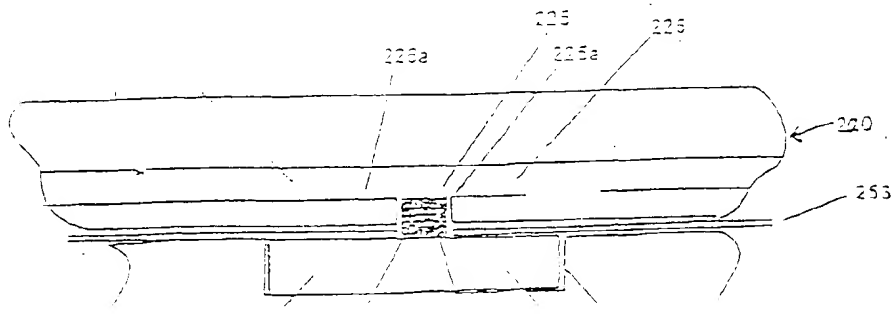
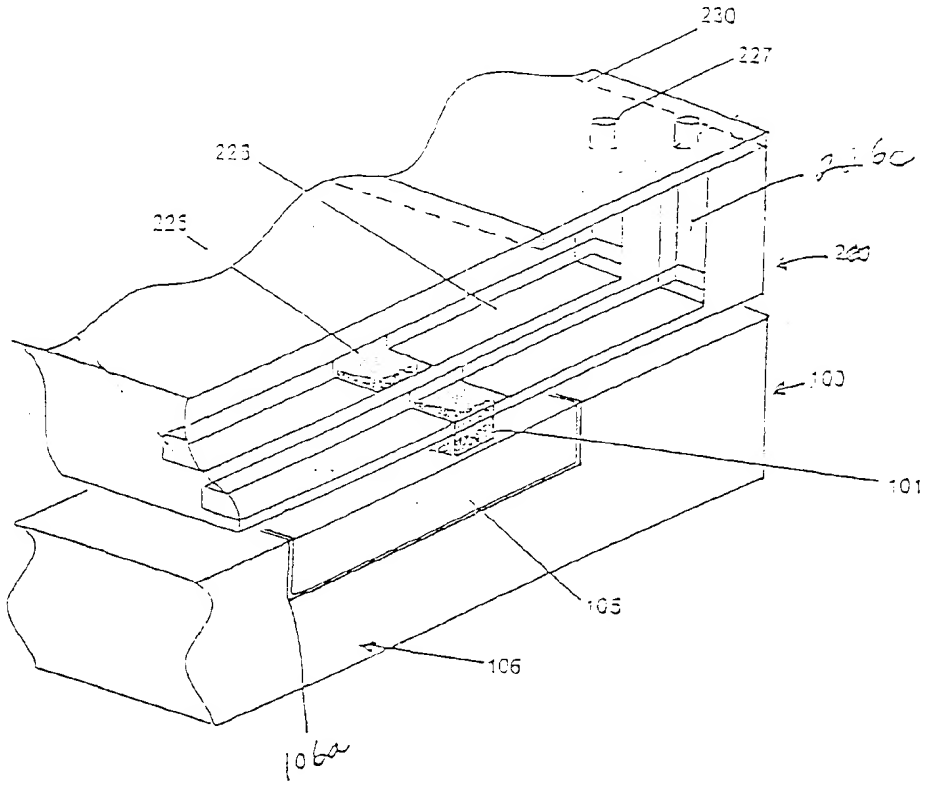
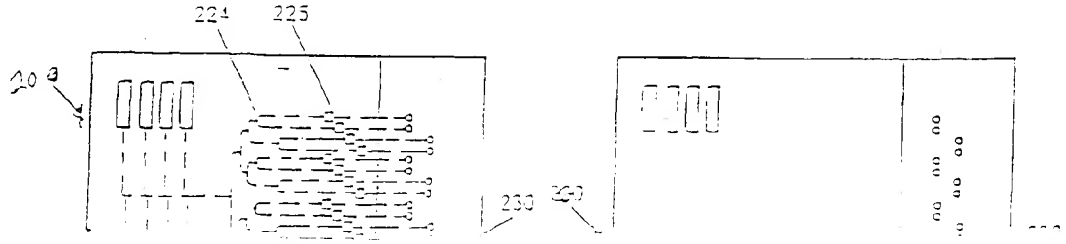

225
226
226a
226a
280
253

FIG. 27





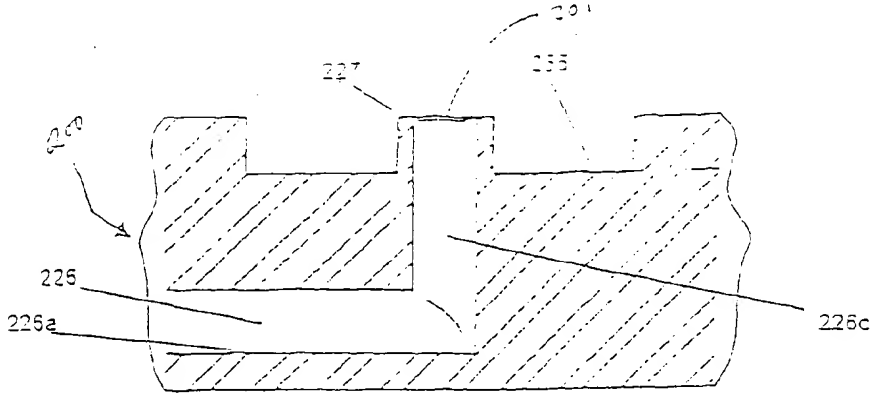

201
227
255
200
226
226a
226c

FIG. 31

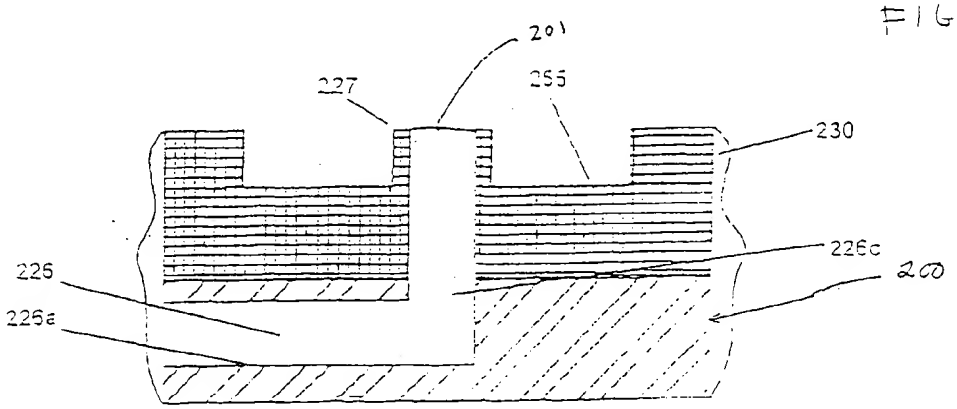

201
227
255
230
226c
200
226
226a

FIG. 32

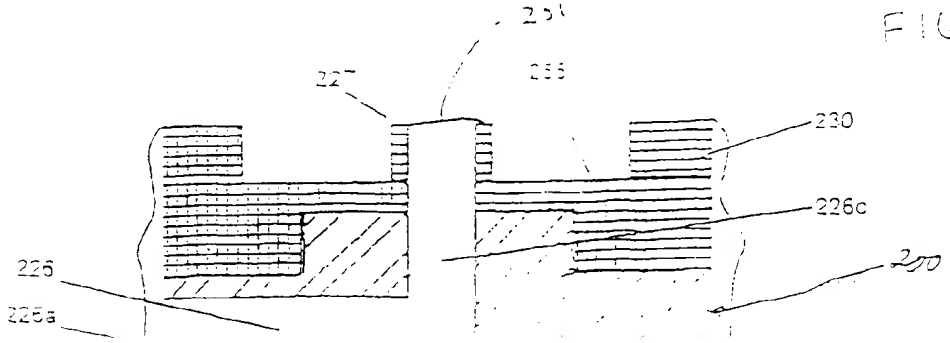

201
227
255
230
226c
200
226
226a

FIG. 33

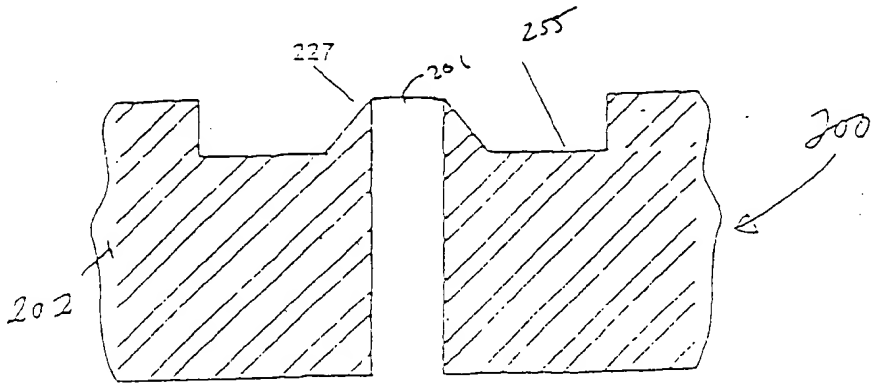


FIG. 34

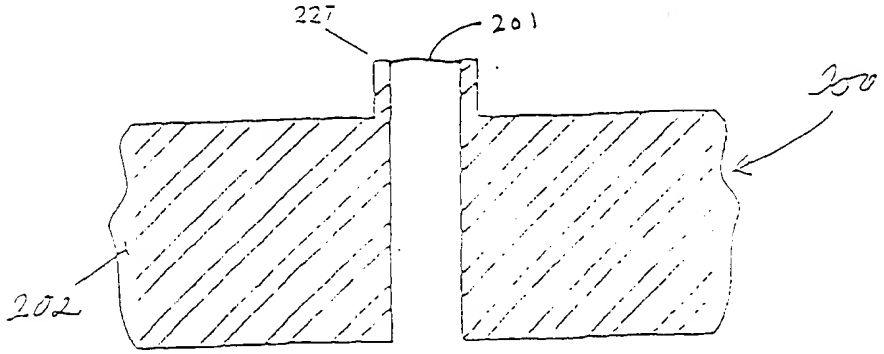
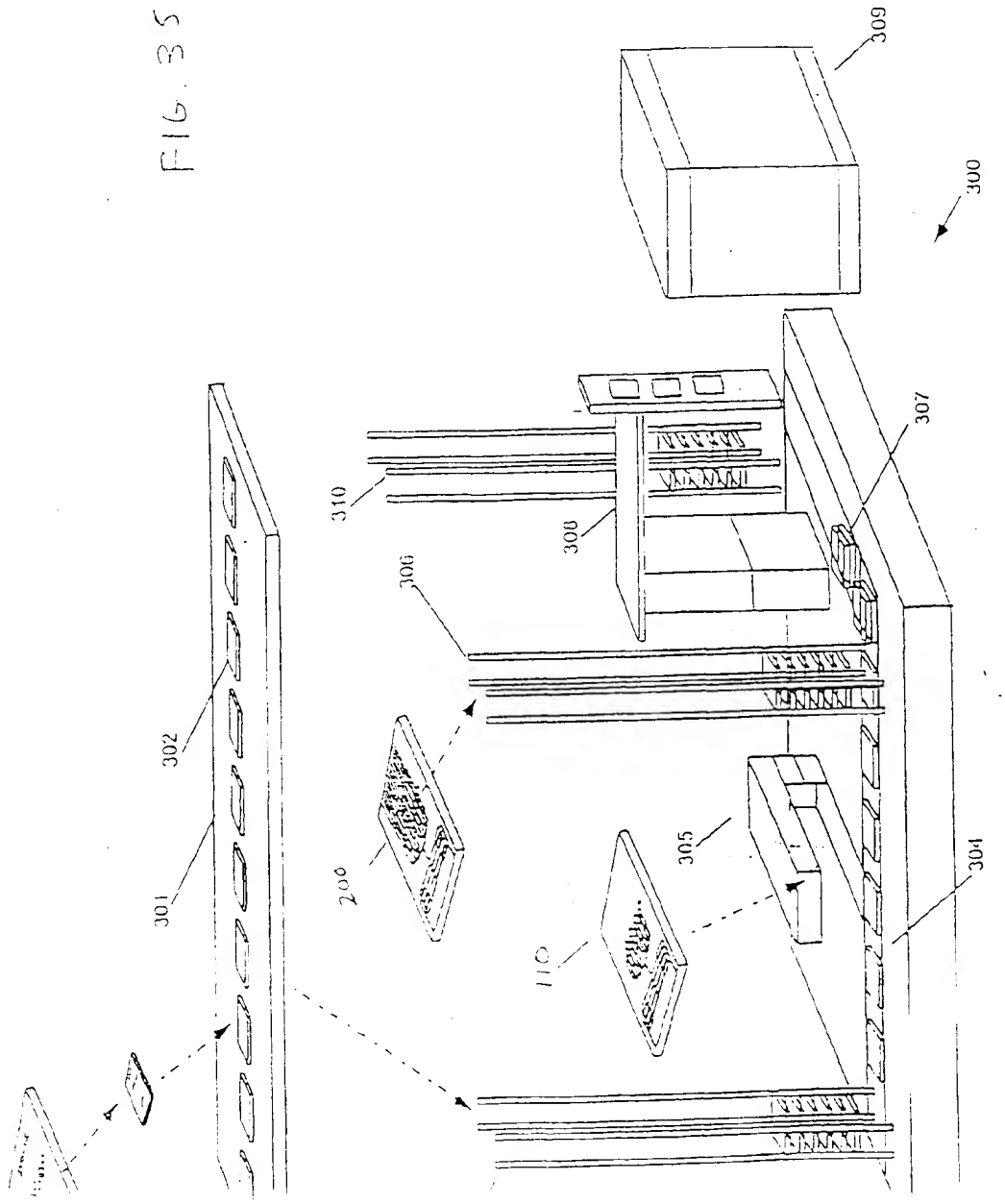



FIG. 35